

nature

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Questioning the quinquennium

THE practice whereby British universities are financed on a quinquennial basis is the root of many of their present problems. In fact, the question now seems to be not so much if, but when, that system will be done away with or modified out of all recognition. The problem is that the arrangements for correcting (for higher-than-expected inflation rates) a particular year's expenditure—planned at the beginning of the five-year period—is cumbersome and drawn out. One of the universities' most immediate complaints, for example, is that their grant for the next academic year, 1976–77, is not yet fixed even though they are already admitting students for courses starting next October and have therefore had to make some fairly detailed plans. One suspects that even the government's much-touted but tentative ideas for introducing cash limits on public expenditure, with no *post facto* corrections for 'extra' inflation, would be welcome, relatively speaking, because at least the universities would then know where they stood.

An added complication, of course, is that the whole business of salaries for university academics is in disarray.

Figures put out recently by the Department of Education and Science show how they have altered, at 1970 prices, over the past five years. The lecturer scale has just about kept up with inflation, and the recent Board of Arbitration settlement means that lecturers' salaries as of October 1974 (which is as far as the negotiations have got!) are some 20% ahead of 1970 in real terms. But senior lecturers and professors, who can hardly be said to earn astronomical salaries, had until the recent settlement fallen seriously behind.

The average professorial salary in 1970 was £5,610, whereas by 1974 it had shrunk a startling 12% to £4,920 at 1970 prices. The level for that year was effectively increased to £5,905 (1970 prices) by the recent settlement. This only put professors about 5% ahead in real terms, and that has been more than eroded in the past year.

Planning anything is difficult for everyone, including the Department of Education and Science. Bearing in mind that academic salaries account for some 50% of university non-capital expenditure, planning is even more difficult when it comes to universities. But is it an excuse for inaction?

Where have all the teachers gone?

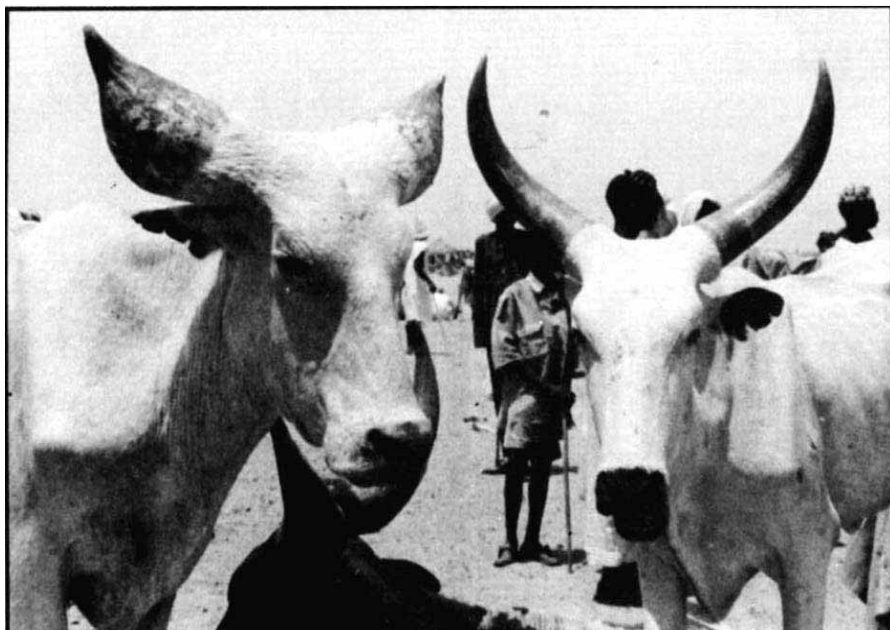
THE shortage of science teachers in British schools seems likely to intensify, with a consequent loss of potential scientists and engineers. This gloomy prognostication emerged recently when educationalists from varied backgrounds gathered at the Royal Society in London to consider problems in the teaching of science and mathematics. The meeting also demonstrated the apparent gap between the aims of many school teachers and the knowledge and attitudes required of first-year students at colleges and universities.

The shortage of scientists and mathematicians in schools, which exists in spite of the overall national sufficiency of teachers, will be exacerbated by the cuts in the numbers and sizes of teacher training colleges, recently necessitated by instructions from the Department of Education and Science. Where small departments within colleges have to be closed down they may well include those concerned with science, so that even fewer primary and secondary school teachers will be available with the background necessary to teach science effectively. Another disturbing factor will be the loss of flexibility in training colleges which are amalgamated with polytechnics and adopt courses leading to the BEd degree of the Council for National Academic Awards. These courses will require students to choose their main subject from the outset, rather than specialising only after a broad, basic course including mathematics and science.

During a wide ranging basic course students can be persuaded, by imaginative teaching methods, that science is not necessarily boring, rigid and too difficult, as they may have thought at school. In these circumstances students might opt to specialise in science or mathematics after the first few terms, but when the choice has to be made at the beginning of the first year the chances are that prejudices acquired at school will have an undue influence.

School teachers who are trying hard to arouse enthusiasm for science and mathematics among their pupils, through the use of new methods and curricula, are apparently coming into conflict to some extent with the needs of colleges and universities. The contributions of academics to the meeting at the Royal Society suggested that students who have learned about theories and concepts at an early age do not all have sufficient factual knowledge when they embark on higher scientific education. It is more important, the academics say, to understand the meaning of *pH* than to be familiar with molecular orbital theory. But the school teachers counter that they have to interest their pupils in science, and this is not necessarily done by constant learning of facts. The teachers ask whether they are supposed to be preparing pupils to enter higher education or to become effective citizens. This is a question that will have to be considered carefully in future deliberations about the structure and content of British education.





Kuri cattle at Oredaya market, Chad

Conserving genetic resources

Many breeds of livestock and innumerable varieties of crops which have arisen in the course of man's agricultural history are now in grave danger of being supplanted by the comparatively fewer highly-bred animals and plants of modern agriculture. A priceless and irreplaceable reservoir of genetic raw material is threatened. Peter Collins looks at efforts now under-way to preserve some of the rapidly vanishing breeds of livestock. Next week Eleanor Lawrence reviews international efforts to conserve crop genetic resources.

CONCERN for the fate of traditional breeds of livestock is an aspect of conservation that has as yet received little attention at the international level. It is true that certain breeds, especially of cattle, that would otherwise have disappeared have been preserved by societies set up for this specific purpose. But most breed societies are at least as much concerned with the quality and performance characteristics of the breeds they represent as with conserving them as sources of genetic material. This is by contrast with the tremendous interest now apparent, and actively fostered over many years by the Food and Agriculture Organisation (FAO), in maintaining genetic stocks of crop plants.

Until fairly recently the specialised breed society was largely a British institution. Although this movement is now spreading, breed societies are still confined principally to the agriculturally more advanced countries, but even they have not prevented the disappearance of a number of ancient and potentially valuable breeds. Nowhere outside the UK is there anything approaching the Rare Breeds Survival Trust, a body specifically concerned with the conservation of British breeds

of livestock that would otherwise become extinct.

Pure stocks of major commercial breeds have, of course, been maintained, especially where their performance is particularly in line with present thinking in the agro-economic field. But the number of such breeds has been declining for many years, and this process is accelerating rapidly. Thus, a recent FAO publication (*Report of a Pilot Study by FAO and UNEP on Conservation of Animal Genetic Resources*, Rome, March 1975) identifies 118 breeds of cattle in Europe and the Mediterranean Basin as being "in a relic state, in danger now, or in danger in the future." It records only 30 native breeds from the same group of countries as "not in danger".

Among those already threatened with extinction are such familiar breeds as the Irish Kerry ("already in a relic state"), and the Red Poll and Beef and Dairy Shorthorns in the UK; even Scotland's Ayrshire is in danger of disappearing. The decline is particularly marked in the dairy breeds, as it is throughout Europe, on account of the tremendous rise in popularity of various types of Friesian. There has

always been a certain amount of deliberate cross breeding in the livestock industry, relying on the maintenance of purebred stocks of the selected parent breeds. This aspect of conservation has scarcely been recognised, mainly because the objective has been increased performance and production of more and better meat and milk, and occasionally, disease resistance.

But it is this point of view that has, for very good reasons, principally influenced the FAO's approach to the problems of increasing livestock production in the developing countries. Thus, the FAO has frequently advocated the use of local breeds in these countries for crossing with introduced animals, but has not been greatly interested in the conservation of other, less immediately useful indigenous breeds. Now, there is increasing concern that the success of such introductions is beginning to erode the irreplaceable genetic resource that exists in traditional native African and Asian breeds. Many of these, it is feared, may disappear before there has been a chance to evaluate their performance under improved conditions, or for cross breeding with other local or introduced breeds.

With the sudden surge of interest in conservation, and more particularly, with the creation of the United Nations Environment Programme (UNEP), this situation has begun to change. UNEP has a specific objective in this field, based on a resolution of the Stockholm Environment Conference of 1972. This is "to initiate the preparation of a comprehensive catalogue of threatened species and varieties of crop plants, fish, domestic animals and micro-organisms, and to cooperate with the FAO in its programme for genetic resource conservation . . ."

Earlier this year the FAO and UNEP published a report of their first joint project. The project—in effect, the first international step towards the conservation of genetic resources of domestic animals—has two objectives. First, "to prepare a preliminary list of breeds of farm animals which are in danger of extinction together with an account of any measures which have been recommended or taken to prevent this extinction"; and, second, an examination of the problems involved in the conservation of a single breed in a developing country.

A breed of humpless cattle known as the Kuri was chosen for the second part of the project. The Kuri, whose habitat is the islands and shores of Lake Chad, is unique in the possession of inflated, spongy horns, apparently (though exactly how is not known) an adaptation to its habits and habitat. A fine swimmer, the Kuri actually feeds

in Lake Chad, eating the coarse forage of the shallow shores, and swims to and fro to reach new pastures. The concern about the survival of the Kuri, however, is based on a series of circumstances affecting, or liable to affect, many other breeds in developing countries.

Since 1960, a rise in the water level of Lake Chad seems seriously to have reduced the available grazing area. Then, because of the demand for Kuri stock, which are described as moderate milkers and excellent beef animals, local farmers could obtain two zebu cattle for one Kuri. This cannot be avoided where numbers of cattle rather than quality is still important to local tribes, but the consequence is dilution of the Kuri herds with zebu blood. In addition, the Sahelian drought of recent years has led to pressure on the Kuri grazing areas by zebu herds from the Sahel.

The original project for the conservation of the Kuri was proposed in 1972 by the Lake Chad Basin Commission. The aim is to establish several herds of pure-bred Kuri, selected with an emphasis on the animals' economic qualities, but including one small herd specifically intended to preserve the unique, inflated, buoy-shaped horns which have attracted many travellers in the Lake Chad region—and which could make these Kuri a tourist attraction in themselves. In its latest form, with UNEP-FAO assistance, this project can be seen as a valuable exercise in the deliberate conservation of a threatened breed.

While most attention has so far been directed towards cattle, the situation is little different where other farm livestock are concerned. Many breeds of sheep, pigs, horses and even goats are in danger of becoming extinct, mainly for the same reasons: changes in the pattern of farming, improvement of land, and the constant demand for higher production based on the use of feeds not available in the 'natural' environment. Other domesticated animals, such as the camel and its South American relatives, have scarcely been considered from the conservation point of view; a minor species such as the donkey could well disappear simply because no one has thought of doing anything to prevent this happening.

In general, there are three aspects of this conservation problem: cultural, scientific and economic. The traditional breeds of livestock are, first of all, as much a part of our cultural heritage as are ancient buildings and works of art. From the scientific point of view, certain breeds have colour genes. The larger species, too, are useful as controllable sources of milk, blood and various tissues of particular genetic interest.

But it is the practical economic argument that is the most important. The classic example of this is probably the "discovery" by American poultry breeders of the traditional Cornish breed, reduced in its home country to a purely fancy bird. Crossed with other breeds, it made possible the rapid growth on which the modern broiler industry depends.

Similarly, remote breeds of sheep, for example from Greece and Finland, were unknown beyond their own homelands until, because they were highly prolific, they were seen to be suited to modern husbandry techniques. There are several very prolific tropical breeds of sheep, too, in the Caribbean, in Latin America, West Africa and South-east Asia, that could also be useful.

An even more important example is probably that of the 'trypano-tolerant' indigenous cattle of various West African countries. If these could be successfully crossed, or developed under improved conditions to provide a more economic yield of meat and milk, it might be possible to avoid the enormous cost of controlling tsetse fly, the vector of trypanosomiasis, which denies thousands of square miles of Africa to the livestock industry.

One apparent problem, however, is the lack of knowledge of the characteristics of most of the indigenous breeds of the developing countries. A first step, which must come before a conservation programme can be set up for any breed, is obviously its evaluation in terms of production, disease resistance, and potential when crossed with other, better known breeds. This means a long and enormously costly series of projects.

Moreover, even where there have been apparently successful cross-breed-

ing programmes, little is sometimes known about what is happening from the genetic point of view. Thus, it was long considered that the "hybrid vigour" (heterosis) apparent when certain exotic, highly developed breeds have been crossed with local stock, was due to the introduced, often larger animal. Now it seems that this is not necessarily the case, and it is essential that sufficient pure-bred herds of the local breed be maintained if such cross breeding is to be continually successful. An example is quoted of the local Criollo cattle in South America, which have been successfully crossed with Brahman (zebu) bulls from the United States. Now the Criollo themselves are in danger of dying out, and the genetic resource they represent could be lost.

Conservation of livestock breeds for economic or cross-breeding purposes demands the maintenance of considerable herds of pure-bred stock, which many of the poorer developing countries may not be able to afford. This therefore is a case for international action. Where a breed is being maintained for scientific purposes, smaller herds, such as might be kept in rare animal parks or large zoos such as Whipsnade, will suffice. Where the interest is solely cultural or historic, such as in the case of the UK's White Park cattle at Cadzow and Chillingham, even fewer beasts need be kept and the problem of cost may be less important than that of management. Certainly, some of the hundreds of breeds of livestock now kept on the world's farmlands will disappear. But with the new approaches to agriculture, and the call to combine higher production with better use of animal and plant resources, everything must be done to ensure that as little as possible of the genetic heritage available in these breeds is lost. □



Kuri cow showing buoy-shaped horns

Relative deprivation of Civil Service scientists

SENSIBLE negotiators will use the breathing space provided by the British Government's £6-a-week pay policy to seek solutions to their long standing industrial relations problems. One such problem is that of the pay and careers of Civil Service scientists, including those employed in research councils, the UK Atomic Energy Authority and other closely related areas of employment.

Civil Service scientists are highly discontented. They have become more frustrated since the hopes raised by the Fulton Report on the Civil Service that equal opportunities would be given to specialist staffs have been seen to come to naught. It is difficult to recall a time in the past 30 years when they have so resented the comparative injustices which they suffer.

Why should this be so? The best point at which to begin is with the recruitment of staff. The Civil Service recruits graduates with a First or Upper Second Class Honours degree—and, perhaps, a second degree—separately to the two main groups, Administration and Science. Although in both cases recruits will meet the academic requirements, there is very little doubt that in practice the scientist will be the more highly qualified.

After a year or two of service the administrator, at every grade level he reaches below £12,000, at which point there is unified grading, will be paid more than his science counterpart. The difference is of the order of 3.5–5.5% at comparable salary maxima. The relationship is similar in respect of A-level entrants to each of the groups.

But this is far from the whole story. The salary differences—themselves impossible to justify and a constant source of irritation—are aggravated further by the fact that career progression in administration is greatly superior to that in science. Consider the example of the high quality graduate, with a First or Upper Second Class Honours degree. The scientist will get to Higher Scientific Officer in his late 20s (only 2.3% are under age 25), to Senior Scientific Officer in his 30s (8.6% under age 30) and to Principal Scientific Officer (£7,205) in his late 30s (0.7% under age 30). A handful will get there more quickly.

Compare this with the high quality

graduate administrator. He will become a Principal (£7,450) between the ages of 28 and 32. It is hardly surprising that he gets there more quickly than most scientists. There is an additional grade (SSO) in the science structure. The administrator will, as a matter of virtual certainty, progress to Assistant Secretary (£11,000) in his early 40s.

Thus the high quality graduate administrator can normally expect to progress to a salary level some £4,000 above that of the scientist. The ratios of promotions above Assistant Secretary (£11,000) for the administrator are broadly similar to those for the Principal Scientific Officer (£7,205) on the science side but, of course, at each stage at one step higher.

The career earnings of the administrator are thus very much more than those of the scientist, as the pattern illustrates. For the situation in which neither the administrator nor the scientist progresses further than indicated—and the chances of doing so are marginally greater for the administrator—a comparison of their career earnings between the ages of 21 and 60 shows that the best administrator will receive 34% more than the scientist. A comparison for average staff shows the administrator with a 24% advantage.

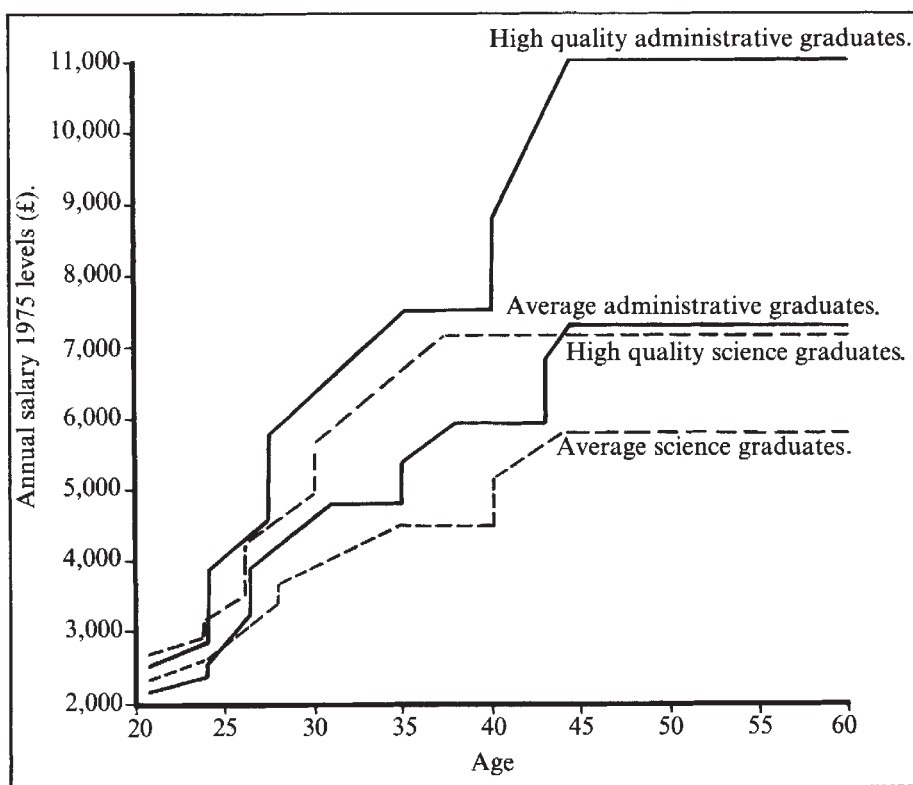
In 1974 there were 1,100 vacancies for science graduates in the Civil Service proper. Posts were offered to

1,415 applicants, but only 661 actually took them up. It is very unlikely that lack of job interest or of good facilities for research were the explanation for this. There can be no doubt that pay and career prospects played a major part in the decision making. In fact, scientists would all be better off financially if they entered the Civil Service as administrators.

Yet, odd as it may seem, very real obstacles are placed in the way of scientists who wish to transfer, later in life, to administration, in spite of the good intentions of the Civil Service Department. In this the Civil Service differs from industry which draws heavily on scientists to fill posts at large.

Civil Service scientists are not obsessed with jealousy of administrators. They do not at all begrudge administrators what they have. But scientists do not see themselves as in any way inferior. They consider that the intellectual contribution of the scientist is worth at least as much by way of pay and career prospects as the contribution of the administrator. They ask no more than that there should be equality of opportunity for all Service recruits of equal ability. Could not all of us agree about that as a reasonable objective? As things are at present scientists are not even in the same race as administrators.

Cyril Cooper



Salary expectation in UK Civil Service

international news

A MAJOR debate is shaping up in the United States over the manner in which new drugs are developed, tested and marketed. Although the issues are hardly novel and the arguments are likely to be shrill, the debate could eventually result in the first sweeping changes in food and drug laws since drug regulations were tightened in 1962 following the thalidomide disaster in Europe.

The focus of the debate will be a bill, drafted by Senator Edward M. Kennedy, which seeks to dismantle the Food and Drug Administration (FDA) and to strengthen and streamline the process by which new drugs are tested and approved. Although the bill has not yet been formally introduced into the Senate, it is already generating considerable discussion.

The impetus behind the bill comes from a series of highly publicised hearings, conducted over the past couple of years by Kennedy's health subcommittee and by a subcommittee on Monopoly headed by Senator Gaylord Nelson, during which the FDA has been flayed from all sides.

On the one hand, a number of scandalous practices in the sale, promotion and testing of drugs have been unearthed, and the FDA has been accused of sloppy, ineffective and even biased regulation of the drug industry. In short, it has been alleged that the American public is being bilked and its health endangered by unsafe and ineffective drugs which have been allowed on the market with inadequate testing and review. The drug industry, which was once widely admired and respected for its contributions to public health has—in some cases deservedly—become an increasingly plump target for criticism.

But, on the other hand, a phalanx of economists, pharmacologists and physicians has argued that the American public is being short-changed for an entirely different reason: the drug laws are now so strict, and the approval process for new drugs is so tortuous, that innovation is being stifled and patients are being denied valuable drugs which are available in other countries, especially Europe.

Whatever the validity of the various charges, it is clear that some changes in drug laws will have to be made. As Mr Kennedy, whose chairmanship of the health subcommittee gives him a pivotal role in such matters, put it during a recent symposium at Tulane

Busting the FDA

by Colin Norman, Washington

University, "there are fundamental defects in our nation's regulatory procedures for prescription drugs, and these defects can no longer be ignored".

Kennedy's bill attempts to bring about a variety of improvements in the situation by breaking up the FDA and replacing it with two separate agencies—a Drug and Devices Administration and a Food and Cosmetics Administration. It also seeks to strengthen the scientific competence of the staff which reviews applications to test and market new drugs; improve and, in some cases, shorten the review process; and establish a system for monitoring clinical experience with new drugs.

The 1962, post-thalidomide amendments to the food and drug laws established a testing procedure which must be followed before any new drug can be marketed in the United States. The goal of the process, which is monitored and licensed by the FDA, is twofold: to determine that drugs meet safety requirements, and to demonstrate that they are effective. The tests, in short, proceed from animal experiments, through limited toxicological tests on humans—usually prisoners—to broader clinical trials to determine effectiveness. The procedure is rigorous and expensive.

Critics of the system who claim that the regulatory filter allows dangerous and ineffective drugs to slip through generally tend to blame the administration of the laws rather than the laws themselves for endangering the health and wallets of the American public. Their central allegation is that the FDA is so heavily dominated by industry pressure that its decisions are frequently biased in the industry's favour. As evidence, they cite the 'revolving door' syndrome in which top FDA personnel are often drawn from the drug industry and usually return to it. And scarcely a month goes by without a Congressional committee hearing evidence that some drug or another is unsafe or useless.

Moreover, the air of public mistrust which hangs over the drug industry doesn't help the industry. The mistrust is understandable, in view of a string

of allegations which have been voiced at the Senate hearings and in various reports and studies.

To take just a couple. Last year, four former drug salesmen told Kennedy's subcommittee in considerable detail of the scandalous gimmicks and hard selling tactics which they used in peddling their wares to prescribing physicians. The tactics ranged from perusing the files of pharmacies to see which physicians were prescribing their company's drugs, to the industry's practice of sending 2,000 million free samples through the mail to physicians each year. And recently, wide publicity has been given to a study, supported by Consumers' Union, which has unearthed a number of instances of drugs which are considered dangerous or ineffective in the United States, being marketed by multinational companies, with limited controls, in Latin American and Caribbean Countries.

But the critics' most widely publicised piece of evidence of alleged industry favouritism within the FDA cropped up in August last year, when 10 present and former employees of the agency testified to Kennedy's subcommittee that they had been harassed or transferred, or that their decisions had been countermanded, when they took action against some drugs. Those allegations immediately brought promises from the Department of Health, Education and Welfare, FDA's parent department, that a thorough investigation of the matter would be conducted. A seven-member panel was eventually established, under the chairmanship of Dr Thomas Chalmers, Dean of the Mount Sinai School of Medicine, to look into the charges. And the FDA also began its own investigation.

The FDA exonerated itself in a massive, 906-page report on the charges last week, but its conclusions were, understandably, not immediately accepted by the critics. The Chalmers committee is expected to complete its investigation early next year, and its report will clearly play a substantial role in the debate on Kennedy's bill.

As for the allegations that the drug laws are so strict that they are stunting the development of new drugs and depriving patients of valuable products, those charges were first seriously raised in 1972 by economist Sam Peltzman of the University of Chicago, and they have since been extended by William Wardell and Louis Lasagna, both of whom are pharmacologists at the Uni-

versity of Rochester. Their studies have recently been published by the American Enterprise Institute, a conservative Washington 'think tank'.

Peltzman examined the rate of introduction of new drugs in the United States before and after the 1962 amendments to the drug laws, and compared his findings with experience overseas. He found that innovation was markedly reduced by the amendments, and he concluded that the net cost to consumers of the delayed introduction or non-availability of new drugs amounts to about \$250-\$350 million a year, over and above any benefits derived from the regulations.

Wardell and Lasagna compared the availability of drugs in the United States with the situation in Britain, where efficacy requirements are not as rigorously enforced, and they concluded that valuable new drugs are marketed more quickly in Britain, and that some drugs widely used in Britain are still not available in the United States.

The FDA's most vigorous and elegant rebuttal of the Peltzman-Wardell-Lasagna thesis was made in 1973 by Dr Henry Simmons, then Director of FDA's Bureau of Drugs, in testimony before Senator Nelson's subcommittee. Citing studies conducted for the FDA by the National Academy of Sciences, Simmons pointed out that of 4,300 drugs marketed before the 1962 amendments came into effect, only 40% showed substantial evidence of effectiveness. He also accused doctors of over-prescribing drugs, and said that adverse reaction to drugs has now developed into a major health problem. It was precisely because of such factors that the 1962 amendments were passed. Simmons also suggested that the FDA has serious reservations about the safety of some drugs that are available abroad, and he cited the lack of any thalidomide-like tragedy in the United States as evidence of the effectiveness of the drug laws.



Senator Edward Kennedy

Kennedy seems to have come to the conclusion that there is more than a grain of truth in the criticisms of both sides. In his speech at Tulane University, he acknowledged that the FDA has some serious problems, but he suggested that they stem from the agency's "incredible range of unrelated responsibilities, and its insufficient scientific expertise". That explains why his bill would cleave the agency into two separate units, each of which would have more manageable responsibilities.

As for the scientific quality of FDA's staff, a host of investigations of the agency carried out over the past decade or so have almost unanimously suggested that the quality needs to be improved. That conclusion is hardly surprising. Because the Bureau of Drugs is spread over six different buildings in Washington alone, there is little opportunity for FDA officials to do research of their own and, as FDA Commissioner Alexander Schmidt recently put it, "opportunities for professional growth are meagre". Kennedy's bill would therefore beef up the quality of the scientific staff by reserving a number of positions for academic pharmacologists to serve in the agency for two or three years, with ample research time and equipment to carry out their own research.

There is, however, at least one aspect of drug regulation in the United States on which most people agree: once a drug is approved and marketed, there is very little follow-up to monitor for unexpected side-effects, or for possible new applications. As Kennedy suggested last week, "the absurdity of the system is staggering. We clear a drug for a particular use. We are meticulous about requiring proof of effectiveness for that use. We spend millions of dollars and years of time developing that proof. But once a drug is finally approved, it is turned loose, free from all controls and follow-up".

FDA does run a system for receiving reports of adverse drug reactions, but a recent report by the Congressional General Accounting Office found the system to be hopelessly inefficient, and even Mr Schmidt acknowledges that "the greatest single weakness of our present regulatory system is the abysmal reporting by almost all professionals of their drug experience—good, bad or indifferent".

Kennedy's bill seeks to take care of those complaints by changing the regulatory process in such a way that some drugs could be more closely monitored and others will be brought more quickly into use. He has proposed that before some drugs are given full commercial approval, they should be placed on a limited distribution to a few hospitals, or to a particular region

of the country, and their use should be closely watched. The proposal would allow drugs which may have potential problems to be closely monitored, and it would also enable products which seem especially promising, and whose safety has been reliably determined, to be brought more quickly into use—limited distribution could be substituted for some of the time-consuming and costly testing for effectiveness.

Mr Schmidt said, also in a speech at Tulane University earlier this month, that the proposal "might permit earlier appearance in the United States of many drugs, in return for a longer investigational phase controlled by FDA. This seems to me a reasonable trade-off". And Dr Wardell said in a telephone interview last week that he believes the proposal has some merit, provided that limited distribution and monitoring of new drugs is not simply added to the already lengthy review process.

As for the suggestion that FDA should be split in two, Mr Schmidt declared himself opposed. The regulation of food and cosmetics, he said, is similar to the regulation of drugs and medical devices, and the two functions can effectively share a central legal and enforcement division. Nevertheless, he acknowledged that "when Senator Kennedy suggests changes in FDA's structure, we are inclined to pay attention".

Kennedy has promised to send his bill to interested parties as soon as it is formally introduced in the Senate, and he will conduct hearings on it next spring. With Congress and the White House both battened down for the elections by then, it is unlikely that the measure will be passed before the end of the year. But the bill will very probably form the basis for legislation in the not too distant future.

● To help pave the way for the science policy office which will soon be re-established in the White House, President Ford has established two top-level committees of scientists to advise the Administration on a number of important science policy issues. One, chaired by Dr Simon Ramo, will "examine issues and opportunities involving the fostering economic strength and in improved utilisation of technology in assuring that economic goals are achieved along with environmental goals", according to a White House announcement. The other, chaired by Dr William O. Baker, will "consider developments that may take place in science and engineering in the decade ahead and examine the national policy implications of these developments". Members of both committees are distinguished and well-versed in Washington science advisory activities. □

THE former President of the Soviet Academy of Sciences, Mstislav V. Keldysh, who retired from his post earlier this year on the grounds of ill-health, seems to have made a recovery, judging from recent articles in the Soviet press. On November 8, the day following the announcement of this year's State Prizes, it was he, and not his successor Academician Vladimir A. Kotelnikov, who gave the traditional appreciation of the achievements being honoured. A few days later, on November 16, he was again featured in *Pravda*, in an extensive interview on the success of the recent Venera 9 and 10 probes.

Neither of the articles is in any way out of context. Keldysh is still Chairman of the Lenin and State Prize Committee for Science and Technology, and was closely involved with the Soviet space programme in its early days. Nevertheless, those who suspected some connection between Keldysh's retirement and the postponement of the Academy's Jubilee Celebrations from May 1974 to October 1975 will doubtless find something more than fortuitous in his sudden reappearance in print so soon after the conclusion of the celebrations.

One interesting feature of this year's State Prize lists is the emphasis given not only to basic research and applied technology, but also to the production of text books. Although in the past Lenin or State Prizes have been awarded to authors in connection with a specific scientific monograph, this year three works intended for students' use have been specifically honoured. They represent a broad spectrum: ordinary differential equations, general surgery, and radio receivers.

The Soviet academic system makes a sharp division between pedagogic and research activities—the universities confine themselves entirely to teaching, while original research is carried out only in special institutes. Although a research scientist will frequently hold more than one post, combining research at an appropriate institute with university lectures, his main chance of official recognition in the State and Lenin Prize lists has until now been through his research.

This new emphasis on the importance of text books is an interesting sidelight on the growing concern of Soviet educationalists who want works of "a high scientific and methodological level" at their disposal for use in universities and colleges of special secondary education.

● Following the recent press campaign against Academician Andrei D. Sakharov, the announcement that

the Soviet government would not permit him to travel to Oslo to receive the Nobel Peace Prize came as no surprise. Nevertheless, the reason given—that he is in possession of classified information—demands some scrutiny. It is more than seven years since Sakharov lost his security clearance, which raises the question of whether any information to which he previously had access is still on the secret list.

Soviet scene

from Vera Rich, London



Moreover, in 1958, the year that Boris Pasternak decided to decline the Nobel Prize for Literature after a prolonged press campaign against him, the award of the Physics Prize to Cherenkov, Frank and Tamm was hailed as a notable tribute to Soviet science, and the three winners received the necessary visas for Stockholm. This was in spite of the potentially sensitive nature of their work: Tamm and Sakharov had actually been working together on certain aspects of thermonuclear reactions.

The Soviet authorities apparently find Sakharov's prize an embarrassment, and for reasons of policy would prefer that he should not receive the award in person. If the precedent is followed in the future, however, a Soviet winner of the Physics Prize might well be forced to receive it *in absentia*.

● Increased concern is being felt for the fate of Andrei N. Tverdokhlebov, the physicist who was arrested on April 18 in connection with his attempts to establish a Moscow group of Amnesty International, and who is now being held in the Lefortovo prison in Moscow. The link between physics and

concern for human rights and freedom dates back to Stalin's time, when the need for a nuclear arsenal allowed physicists to speak from a relatively privileged position and to avert a Lysenko-style disaster in the physical sciences. Tverdokhlebov has been closely associated with Academician Sakharov, whose "Human Rights Group" he joined as a founder member, and also with Valentin Turchin, who was a co-signatory of the second Sakharov manifesto of 1970. An appeal by Turchin on behalf of Tverdokhlebov has been sent to the West. According to press agency reports, Tverdokhlebov and another member of the Amnesty group, the Ukrainian science-fiction writer Mykola Rudenko, will be charged with "anti-Soviet slander", but it is not yet clear whether formal charges have been made.

● Two significant developments in the field of automation have been announced recently in the Latvian SSR. The first, described at the First All Union Conference on Biomechanics in Riga, is a proposed walking robot for rough terrain. Although this has been mooted several times in the last few years, the new version, designed on the basis of extensive mathematical simulation, seems to have been produced with far greater attention to practical implementation than some of the rather theoretical proposals so far discussed. The latest ideas include lasers as a possible means of stimulating vision, and the automatic selection of the form of motion—velocity, length and height of steps—to fit the demands of the terrain. A six-legged robot is likely to prove the most manoeuvrable.

The second and perhaps more alarming development concerns the management-control automation system recently introduced into the radio industry in Latvia. According to the *Novosti* agency, this system, based on a Minsk 22 computer, is intended to assess the competence of production managers.

Among other things, it checks their actual performance against planned deadline dates, and acts as a progress chaser by sending telex messages to remind managers of their obligations. At the end of the month, it evaluates the manager's place in the hierarchy of professional "emulation" and adjusts his pay accordingly.

The system, which operates with a staff of five, is said to repay the investment costs within a year by reducing inter-office correspondence by some 50%, as well as improve labour discipline. Its adoption has been recommended for all radio-engineering enterprises in the USSR. □

Another carcinogen?

from Laura Swaffield

THE problems of carcinogens in industry (see *Nature*, 258, 94; 1975) are again highlighted by an article in the journal of the British Society for Social Responsibility in Science. The substance now implicated as a carcinogen is trichloroethylene, widely used as a degreaser and chemically similar to vinyl chloride monomer (VCM).

Its alleged link with humans will be harder to prove than with VCM, which is said to produce a rare form of cancer (angiosarcoma). And whatever the eventual findings, enforcement of controls may be difficult. Trichloroethylene is an important cause of gassing and is well known already as a narcotic with dramatic effects.

Precautions are laid down in the UK, though they are by no means always met, and it remains debatable whether enough attention has been paid in the UK to its longer term effects. □

Continuing crisis in Italian universities

from Gillian Boucher, Venice

As the *Corriere della Sera* put it on November 5, "Today the universities are opening: Why?" The article, concerned with the colossal extent of graduate unemployment in Italy, touched on only one of the problems of the universities—problems that seem so daunting and so far from being soluble by the universities themselves that one marvels to see concerned professors still perfectly sane and conducting themselves in a manner not dissimilar from that of their colleagues in more fortunate countries.

But with the universities open to all who have passed the state school-leaving examination, laboratory and library facilities for teaching are hopelessly inadequate. It is not uncommon for professors to cope with their swollen classes by giving the same lecture three times in a morning. A much tougher entrance examination, even if it were politically acceptable, would not be a fair way of selecting students for science courses because of the bias of the school curriculum: Petrarch and Dante loom larger than science for the average Italian school-child.

Many university teachers would welcome instead a tough examination at the end of the first year of university, but even that smacks too much of elitism to be politically acceptable at present. Fortunately many students drop out voluntarily. Last year at the

University of Padua there were 2,500 first-year medical students, 1,000 in the third year and 500 in the sixth, final year.

Medical schools are in a particularly difficult position, for they have to compete for medically qualified staff with hospitals, which pay far better. The large pay increases recently awarded to hospital doctors mean that university departments often have to fill their junior posts with people lacking medical qualifications, further increasing the teaching load of medically qualified professors.

Difficulties in recruiting first-class graduates are not confined to medical departments. The chief problem is that since 1973 there have been created no more *assistenti*—junior lecturers waiting for a chair who have a certain amount of security and who consider themselves definitely part of the establishment. Now *assistenti* are being replaced with *contrattisti*, who have only a four-year contract and no certainty of a university career beyond that.

Restructuring at the top of the ladder is causing perhaps even more of a headache. The 3,000 top professors (known as *professori di ruolo*) have, with the growing student numbers, been aided by more and more assistant professors, or *professori incaricati*. Although this arrangement was obviously cheaper for the state, in 1973 it was finally decided that 7,500 new *professori di ruolo* should be created by competition: 2,500 in 1973–74, 2,500 in 1974–75, and a further 2,500 in 1975–76.

In the competition, a candidate's publications are assessed by a committee of *professori di ruolo* in the candidate's field, the committee's decision being subsequently blessed by the Ministry for Public Instruction and the university concerned. Unlike previous competitions of this sort, in which the committees used to be elected by all the *professori di ruolo* in a field, the names of the members of the committee are now picked out of a hat by the Minister for Public Instruction. This system has not prevented unconscionable delays in the formation of the committees—mostly caused by obstructive *professori di ruolo* who did not wish to see their privileges diluted by numbers.

The final and fundamental problem is that of money. Once the ministry has spent what it considers necessary for school and university teaching, the sum remaining for research is usually enough to pay for the animal houses and the telephone bill. University research is saved only by the Consiglio Nazionale delle Ricerche (CNR) which, as well as supporting autonomous institutes, funds institutes in university departments and provides grants for individuals.

Anyone lucky enough to be funded by the CNR can feel comparatively secure until 1980, the end of the coming quinquennium. But the CNR is moving towards a Rothschild-like attitude to research for the consumer and may become unwilling to fund projects which fall outside its own guidelines. If a new body being canvassed at present, a Ministry of Scientific Research, is ever created, the CNR would probably become its executive branch. Then, with research firmly separated from education, the CNR would no longer fund research in universities. And that would be the death of science in Italian universities. □

Eurospace body acts

from Angela Croome

It hasn't taken long for the recently formed European *ad hoc* Committee for Space Science to go into action. Just a week after the European Science Foundation in Strasbourg agreed to its formation, two members of the committee attended a Washington meeting of the Space Sciences Board of the US National Academy of Sciences—the committee's American counterpart as a clearing house for long term research policy in the space sciences.

Active subjects on which high level international planning and cooperation are sought include the proposals for an International Planetary Decade being sponsored by the International Council of Scientific Unions' committee for space research, COSPAR, and the project for a large space telescope as an international research facility. A joint meeting of the American board and the European Committee on the telescope is expected before long.

There is likely to be a good deal of overlap between both the business and the membership of the European committee and a similar committee formed to concern itself with astronomy—though the latter has an initial lifetime of only a year, whereas the space sciences committee is to operate for two years.

The newly formed committee will want to keep an eye on the European Space Agency, established earlier this year, most of whose funds have gone on technology projects, principally satellite applications, rather than scientific research. The agency itself accepts that the balance is not right at present.

In the UK, meanwhile, the Director of Jodrell Bank, Sir Bernard Lovell, has said that the large area interferometer system financed through a £1.9 million grant from the Science Research Council is unmatched in its scale and sophistication. He also sees it

WHEN in 1947 I went to Nigeria to try to start that country's first university at Ibadan, I had a capital sum of £1.5 million provided by the British government for buildings. Tough negotiations persuaded the Nigerian government to increase its proposed annual grant (to meet other costs) from £60,000 to £100,000. These sums soon proved to be inadequate and larger amounts eventually became available; but until oil was discovered in the country we were always short of money.

I therefore find it hard to be as sympathetic as I should to the universities of the UK in their present financial position. After all, their grant from the government has increased by some 200 times in 25 years. I think that it is right that their expenditure should be carefully scrutinised.

There is growing concern about student numbers and the entry into different faculties. Politicians and others have suggested that money should not be spent on training specialists for jobs which do not exist, especially when the training is particularly expensive. Unfortunately, attempts to relate graduates to jobs have seldom been successful. Thus in medicine we had the Willink and Goodenough committees, which restricted the growth of medical schools at a time when expansion was later shown to have been needed.

Yet this should have been a comparatively easy exercise for, notwithstanding the egregious mistakes of our demographers (who in 1945 estimated that by 1965 Britain's population would fall by 4 millions, though in fact it increased by about the same number), the number of patients and their diseases remains fairly constant year by year. Few people would suggest at present reducing our intake of medical students, even though it costs the taxpayer something like £100,000 to train a qualified doctor.

Universities claim, with some justification, that they need to maintain a good academic balance, and that one faculty should not grow so rapidly that it overshadows the others. Some even believe that there is an ideal ratio for the number of students in different faculties. I remember hearing Lord Hailsham,

when in charge of higher education some twelve years ago, deploring the insistence of universities on enlarging their faculties of law and social science, even when there was no increased demand for lawyers and social scientists, as a condition for producing the larger number of scientists for whom there seemed, at that time, to be a demand. Sadly,

Lowering costs in higher education



KENNETH MELLANBY

some of the increased number of scientists found, after graduation, that they were also unwanted. To estimate future manpower needs is clearly difficult.

When funds are plentiful, there is less demand to regulate entries into expensive courses. We are rightly proud of our high academic standards, and resist attempts to lower them. Nevertheless more thought could be given to reducing costs. If we are admitted to a British hospital, we will probably be attended by a doctor who qualified in India or Pakistan. Some may arrive with inadequate English and others (like, unfortunately, some of our own graduates) may be incompetent. But the majority carry out their duties admirably. Yet the cost of their training was only a fraction of that incurred in the UK. It is difficult not to believe that there is some room for economy, even in medicine.

Veterinary training is not quite so expensive, but costs probably amount to about £50,000 a graduate. This is justified because the veterinary sur-

geon makes an important contribution to food production by his care of farm animals. The present trend will double the number of vets in practice in Britain within the next 20 years. It is right to wonder whether this is wise or prudent. At present more than half of veterinary practice is devoted to what are, rather charmingly, called 'companion animals'. These are pets, dogs, cats and also horses and ponies. Now it would be wrong to suggest that these are unimportant. They bring pleasure to many, and companionship and solace to elderly and lonely people. They need to be cared for.

But it is surprising to find that some 6 million dogs, rather fewer cats and 250,000 horses take up more veterinary time than do 50 million farm animals (cattle, sheep and pigs) and nearly 150 million poultry. If our farm animals are receiving anything like proper attention, some companion animals would seem to be overprivileged, particularly as experience suggests that the only contact many dogs and cats have with a vet is to be put down when they are old or unwanted. If, as many expect, the number of intensively kept farm animals falls substantially as supplies of cheap imported feeding stuffs dry up, will we need as many vets for agriculture? Can we afford them for our pets?

It is generally agreed that all our school leavers able to profit from higher education should have the opportunity to do so, but need this be so expensive? Can we really justify a staff-student ratio three or more times as high as that obtaining in many other countries? Has enough attention been given to devising more general—and less expensive—courses? We often hear that change in science-based subjects is so rapid that every practitioner will have to be retrained at least once every 10 years. Are we devoting enough of our scarce resources to retraining, and might it not be better to reduce the scale of that given initially?

No doubt these problems are in many people's minds, but so far changes have generally raised rather than lowered academic costs. Perhaps the financial crisis will ensure that action is taken to reduce waste and still to maintain standards.

as an important step towards the 375-foot radio dish planned for the observatory's Meiford site in Wales, some 85 km away.

The new facility, which will use three existing radio dishes that are capable of working down to centimetre wavelengths, will also increase the inter-

ferometer baseline from 20 to 85 km by adding a new 25 m low cost dish in Montgomeryshire. The whole system will be centrally controlled from Jodrell's new interferometer control room.

"The new facility will not replace the 'big dish'—it simply makes the best

use of the equipment and expertise we already have", says Sir Bernard. As for the 'big dish', he still hopes to finance the 375-footer at Meifod. Plans had to be dropped two years or so ago because the international tenders were far higher than expected: £16 million as against an estimated £10 million. □

correspondence

Computer prehistory

SIR,—Professor Wilkes (October 16) identifies the prehistory of computers with “the life’s work of just one man, Charles Babbage (1792–1871)”. If Babbage’s work is likened to the prehistory of the subject, how is one to classify the elaboration of the real first arithmetical machine, almost two centuries earlier, by Blaise Pascal?

Pascal invented and built his arithmetical machine in 1643, at the age of 19, and obtained a patent for it (*Privilege pour la Machine d’Arithmétique de M. Pascal*) in 1654, at which time he had already constructed more than 50 different models. Although Babbage wrote in rather denigrating terms of “the contrivances of Pascal and others” (which actually worked) as compared to his own engines (which did not), Pascal’s invention was indeed fundamental, and the actual precursor of all mechanical computers, including Babbage’s.

A number of Pascal’s machines are still in existence, for example, at the Conservatoire des Arts et Métiers in Paris. A detailed description of the mechanism and principles of Pascal’s arithmetical machine was published in 1751.

C. J. VAN OSS

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EEC directives

SIR,—As a member of the Conservation Society since its inception, and being much concerned about pollution, I would like to support Eric Ashby (October 16) against Paul French (November 6). The latter may be right in his final statement to the effect that other Europeans are more willing to tackle pollution than are the British. As a result of their efforts, for all I know, the Rhine may now, like the Thames, have more fish in its lower reaches than it has had for 150 years.

But his insistence that identical risks should apply everywhere is absolutely unreasonable. In the denser parts of big cities a complete ban on the burning of smoky fuels has reduced by several times the level of smoke pollution. It would not have been sensible to apply the same rules to villages and isolated farms in the country. Not only would uniform laws for town and country have led to a large extra cost for little useful achievement, but, much more seriously, such laws would never

have been passed. I do not believe that in any country in Europe costly and novel proposals, however valuable in the long run, will fail to arouse some opposition. If they can be shown to be technically unreasonable over large areas, opposition will be much greater.

I can see that administrators like to have a uniformly applicable formula, against which performance can be judged. The new formula, which says that pollution is equally important everywhere, is a real advance on the previous non-formula which in effect said that most pollution was of no importance whatever.

It would not seem any more difficult, however, to use a formula which recognised that pollution is more important if more people are polluted. One would do better with a formula based on the square of this number, since if an area has twice as many people they are likely to produce twice as much pollution and there are twice as many to suffer this double pollution. A linear system (the importance of pollution being finite for zero population) would be much easier however, as the standard effluent permitted in any area could then be fixed only in terms of the slowly changing population in the affected area and not at all by the statistically larger fluctuations of the numbers of polluting factories.

Like Mr French, I would be entirely in favour of Britain spending £100 million a year on reducing pollution (and simultaneously, perhaps, unemployment). But to spend this on reducing pollution in the Atlantic Ocean rather than in the thousands of kilometres of rivers in which no fish can live would be a technical and economic stupidity that has nothing to do with politics or nationalism.

JOHN H. FREMLIN

University of Birmingham, UK

SIR,—I am not prepared to accept the whole of Lord Ashby’s thesis, contained in his comments on the EEC pollution debate. He states, for instance, that “rabid conservationists need to be reminded sometimes that all pollution, except that from atomic weapons, is a by-product of processes which benefit society.” I would have thought that conservationists—rabid or otherwise—are merely saying that any process which endangers the environment for future generations cannot possibly be regarded as beneficial to society.

Again it seems to me complacent, to

say the least, to accept a report published by the Medical Research Council 16 years ago as a justification for pouring more sewage on to our beaches and noxious chemicals into our rivers, even though myriads of microbes may be working late into the night to lessen this evil.

Lord Ashby also refers to the phoney arguments. What can possibly be more phoney than his own argument that a cost of £100 million to reduce pollution is prohibitive when we spend many more millions every year on luxuries such as gambling?

W. J. DAVIES

Tenby, UK

Offshore structures

SIR,—We have had some experience with modelling offshore structures in the laboratory, and wish to call attention to the need for better understanding of the engineering problems likely to arise as drillings are made in deeper and deeper water. Mishaps that have already occurred have been serious enough, but the failure of one of the giant rigs or platforms now being planned would be a catastrophe of unprecedented proportions.

Waves not only exert the major load, which is horizontal and fluctuating, but they also affect the sea bed under and around the structure. The interaction is complicated and difficult to model, but studies and fragmentary prototype data have provided some clues. The foundation and anchorage problems assume greater importance as structures grow in depth.

Although some research seems to be in progress, there is little doubt that it has only just commenced. The present programmes are carried out for private industry whereas research on hydraulic phenomena was formerly for government agencies. Past results were thus public, but the present research is mainly *ad hoc* and confidential, in the long term being more costly to all concerned. Reasons for mishaps now tend to be kept secret for political or commercial reasons.

Basic issues should be investigated openly by university and similar institutions, if only so that controlling authorities and insurance agencies can establish design criteria.

CHESLEY J. POSEY

University of Connecticut

RICHARD SILVESTER

University of Western Australia

news and views

Histocompatibility antigens and immunoglobulins?

from Alan F. Williams

In higher vertebrates there is a chromosomal region which contains closely-linked genes for histocompatibility antigens, and also genes which regulate immune responses. The major histocompatibility antigens which are recognised by antibody are called H-2 (K and D) and HLA (-A and -B) antigens in mice and men respectively, and seem to be coded for by two loci which are polymorphic within a species. The function of histocompatibility antigens is unknown, but the finding of genes controlling immune function in the same chromosomal region has led to the idea that histocompatibility antigens may also be involved in tissue interactions as cell-surface receptors. Taking this idea further, it has been suggested that these antigens may be related evolutionarily to immunoglobulin (see Bodmer, *Nature*, **237**, 139; 1972; Schreffler and David, *Adv. Immun.*, **20**, 125; 1975, for reviews).

Recent biochemical studies on histocompatibility antigens of mouse, man and rat have shown that they are carried by the polypeptide chain of a cell-surface glycoprotein of about 45,000 molecular weight, and that closely associated with this, but not covalently bound, is another polypeptide of about 12,000 molecular weight which is not polymorphic. This latter molecule is β_2 -microglobulin which shows amino acid sequence homology with immunoglobulin (see *Transplant Rev.*, **21**, 1974). These findings have been interpreted as supporting the idea of a receptor function for histocompatibility antigen, and also the possibility that the 45,000 glycoprotein is related to immunoglobulin. In fact such is the euphoria which has been engendered, that virtually every product of genes linked to the histocompatibility antigen region is now being considered to be related to immunoglobulin (for example Capra *et al.*, *J. exp. Med.*, **142**, 664; 1975). This all involves a number of extrapolations and it is relevant that neither immunoglobulin nor β_2 -microglobulin genes are linked to the histocompatibility antigen region (see the reviews cited above and Goodfellow *et al.*, *Nature*, **254**, 267; 1975).

It is obvious that the relationship between histocompatibility antigen and immunoglobulin can only be determined by comparison of amino acid sequences, but in lieu of such data more circumstantial similarities have been sought. Several workers (Strominger *et al.*, *Transplant Rev.*, **21**, 126; 1974; Cresswell and Dawson, *J. Immun.*, **114**, 523; 1975; Peterson *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 1612; 1975) have suggested that histocompatibility antigens are composed of a four-chain structure of two identical 45,000 glycoprotein molecules plus two molecules of β_2 -microglobulin linked by disulphide bonds between the large molecules. This is based on the apparent size of histocompatibility antigen in detergents determined from their hydrodynamic properties, and also by the finding of bands in SDS polyacrylamide gel electrophoresis which appear to be dimers of the large subunit. Other evidence cited as establishing a link between histocompatibility antigen and immunoglobulin is that the antigens may contain intrachain disulphide bonds like those in immunoglobulin, and that histocompatibility antigen has affinity for a bacterial product—staphylococcal protein A, which binds to immunoglobulin. One might describe the evidence for all these points as tentative, and interpretations relating to the size of the antigens in detergent may be incorrect. Thus Snary *et al.* (page 240 of this issue of *Nature*) have produced evidence suggesting that the molecular weight of HLA antigen in deoxycholate is incompatible with a four-chain structure.

There are difficulties in estimating the size of membrane molecules in non-dissociating conditions. Integral membrane proteins are usually solubilised by detergents of which they bind large amounts (Helenius and Simons, *Biochim. biophys. Acta*, **415**, 29; 1975). Tanford *et al.* (*Biochemistry*, **13**, 2369; 1974) have described methods for accurate estimates of molecular weight in detergent and from chemical composition, measurements of detergent binding, and sedimentation equilibrium

data, molecular weights can be calculated. For this, milligram amounts of pure material are needed while at present, estimates of the size of histocompatibility antigens can only be made with detergent extracts of impure material. Under these conditions only the size of the detergent-antigen complex can be estimated. This is derived from measurements of Stoke's radius by gel filtration, and sedimentation coefficient and partial specific volume ($\bar{v}$) by zone sedimentation on sucrose gradients in H_2O and 2H_2O (Meunier *et al.*, *FEBS Lett.*, **24**, 63; 1972; Clarke, *J. biol. Chem.*, **250**, 5459; 1975). Analysis by gel filtration and sedimentation on sucrose gradients in H_2O is a minimal requirement and large errors will result, particularly if gel filtration only is used since most membrane proteins including cell-surface antigens behave as if asymmetric in detergents (Letarte-Muirhead *et al.*, *Biochem. J.*, **143**, 51; 1974; Snary *et al.*, *op. cit.*; Clarke, *op. cit.*). A $\bar{v}$ value is often assumed rather than measured, and this is reasonably valid if the detergent used is deoxycholate since this has a $\bar{v}$ of 0.76 ml g^{-1} , close to that of proteins and glycoproteins ($0.7\text{--}0.75 \text{ ml g}^{-1}$). With non-ionic detergents whose $\bar{v}$ is about 0.9 ml g^{-1} a valid assumption of $\bar{v}$ cannot be made without detergent binding data.

Snary *et al.* solubilised HLA in deoxycholate and carried out gel filtration and zone sedimentation on sucrose gradients; a $\bar{v}$ value was assumed. From this, molecular weights which include bound deoxycholate of 88,000 and 78,000 respectively were obtained for A and B series HLA antigens. Similar data has been obtained for rat histocompatibility antigen in deoxycholate and the molecular weight was estimated to be 100,000 (Letarte-Muirhead *et al.*, *op. cit.*). These values are more compatible with a two-chain structure plus bound detergent (minimum size 57,000), than a four-chain structure (minimum 114,000). Peterson *et al.* (*op. cit.*) estimated the molecular weight of HLA and H-2 in the non-ionic detergent NP-40 to be 130,000. This would be compatible with a four-

chain structure with little detergent bound, or more likely a two-chain structure plus a large amount of bound NP-40.

If the antigen-detergent complex does not contain a four-chain structure then there is the difficulty of explaining the reason for the appearance of the 45,000 chain in SDS polyacrylamide gel electrophoresis. Snary *et al.* have suggested that this may be due to disulphide exchange either in stored membrane, or possibly during immunoprecipitation procedures. Clearly the experiments must be repeated in the presence of oxidising agents.

If one accepts that histocompati-

bility antigens may have a two-chain rather than a four-chain structure does this argue against a relationship between the antigens and immunoglobulin? Obviously not, and this merely points up the circumstantial nature of the current evidence. The matter can only be settled by amino acid sequence analysis of the 45,000 molecular weight glycoprotein which carries the antigens, and comparison of this with immunoglobulin sequences. Given the current progress in the purification of HLA and H-2, and also in microsequencing techniques, such data should be forthcoming in the next few years. □

Chemical language in insects

from our *Insect Physiology Correspondent*

It has long been known that scents produced by glandular organs play an important part in animal communication. The naming of such chemicals, used for communication between members of the same species, as 'pheromones' by Karlson and Lüscher coincided with the development of gas liquid chromatography, mass spectrometry and other methods which have made possible the chemical analysis of minimal quantities of material. The result has been a very rapid expansion of knowledge in this sphere. Whereas it took Butenandt and his associates many years and vast amounts of material to identify the chemical structure of 'bombycol', the female sex attractant of the silkworm moth, by classical procedures, complex mixtures can now be analysed in a matter of days.

The chemicals present in the glandular secretions of insects are often exceedingly diverse; 20-30 recognised components in a single gland. They comprise a vast range of saturated and unsaturated hydrocarbons and their derived alcohols and aldehydes (ranging from hexenal and tridecene to pentadecane), fatty acids (such as butyric and caproic acids), a great number of phenolic substances and quinones (such as phenol, *m*-cresol, benzoquinone and toluquinone) and a variety of terpenoids (such as geraniol, farnesol, citral, and citronellol).

So far as the perceptions of man are concerned, in recognising the odours characteristic of particular kinds of shops, or appreciating the bouquet of a vintage wine, it seems to be the complex mixture which is assessed. Insects seem often to be concerned with a single component: in the mandibular gland secretion of the queen honey bee (as shown by Butler and his associates) there are more than 30 compounds; of these, 9-ketodecenoic acid is the

'queen substance' which attracts males during the nuptial flight and inhibits royal-cell building by workers in the nest, whereas 9-hydroxydecenoic acid causes clustering and stabilisation of worker swarms.

The action of pheromones is commonly divided into two classes (following Wilson and Bossert): chemical 'releasers' of specific acts of behaviour, and 'primers' which seem to act initially on the endocrine system. The same pheromone may show both types of activity—as in the case of the queen substance which, besides its effects on behaviour, inhibits ovarian development in the workers. Moreover the same chemical can be used both as a pheromone for communication within the species and as an 'allomone' (as defined by Brown and Eisner) to provide defence against an enemy. That combination is well seen in the use of formic acid by the wood ant. Thus the effects of a pheromone depend on circumstances, on differences in concentration, and in some instances on the combination of secretions from different glands. It has even been suggested by Bossert that concentrations might be modulated in time. It is unlikely that the modulation of odour emission could attain the level of a symbolical or syntactical language but as E. O. Wilson points out the acoustical system has acquired quite complex signal modulation in the songs of insects.

The signal provided by a pheromone is the 'active space' as defined by Bossert and Wilson: the zone around the point of emission within which the concentration of pheromone molecules is above the threshold level of response. This zone may vary in shape with movement of the air, and in composition depending on the comparative rates of diffusion of the varied molecules. As Wilson has pointed out "the relation

of chemosensory physiology to ecology can be fully elucidated only through the analysis of the active spaces".

These ideas have been carried some steps further by interesting observations of Bradshaw, Baker and Howse on the multicomponent pheromones of the weaver ant *Oecophylla*, as described on page 230 of this issue of *Nature*. These authors find evidence of caste differences in the mandibular gland secretions as well as differences in the components to which the castes respond. Furthermore, they have made interesting advances in studying the changes in the 'active spaces' of particular components which appear to provide sequential messages determined by the volatility of materials and the threshold concentrations for different responses. Worked out in detail in relation to the mode of life of a polymorphic species, all this shows the immense potentialities of chemical communication in the regulation of behaviour in a social insect. □

Fibroblast model for hormone effects

from Robert Shields

ONE of the most striking features of the action of trophic hormones on cells is that many of the resulting changes in metabolism are remarkably similar, regardless of the type of hormone or the target tissue. Thus changes produced by oestrogen in the uterus, corticosteroids in the liver and insulin in muscle are much the same. These changes include alterations in macromolecular synthesis and a great deal of endocrinological research has been directed at finding out how hormones regulate these processes. The classical endocrinological approach has been to remove the gland producing the hormone under study and after a suitable period re-add the hormone and follow the subsequent changes in the target tissue. Although much qualitative data has been gathered in this way, the lack of suitable *in vitro* systems has hampered a quantitative assessment of the inter-relationship between the hormone-induced changes in RNA, protein and DNA synthesis.

One well known *in vitro* system that has been studied intensively (but for rather different reasons) is the control of growth of fibroblasts in tissue culture. It now seems apparent that the growth of these cells is regulated by the levels of serum factors available to the cell (*News and Views*,

253, 689; 1975) and that these factors are probably hormones which have not yet been fully characterised. Decreasing the availability of serum to cultured cells either by reducing the serum concentrations or allowing the cells to reach confluence is the *in vitro* equivalent of removing the gland producing the trophic hormone, re-adding the serum is the equivalent of hormone replacement. Since the changes in metabolism induced in serum-stimulated fibroblasts are very similar to those produced by trophic hormones (Hershko *et al.*, *Nature new Biol.*, **232**, 206; 1971), a study of the changes induced in these cells may give clues as to how trophic hormones operate *in vivo*.

Rates of macromolecular synthesis are almost invariably assayed by incorporation of radioactive precursors, and the accuracy with which the incorporation rate reflects rates of synthesis is open to question. An alternative indirect approach depends on assaying steady state concentrations and rates of degradation of macromolecules. The inferred rates of synthesis can then be compared with those from tracer studies. By this method it has been deduced that growing fibroblasts have about four times the protein synthetic rate per unit protein than non-growing cells and differences of this order of magnitude have indeed been suggested by labelling studies (Rudland, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 750; 1974). In principle this approximately four-fold increase in protein synthetic rate could be produced by controlling the levels of cytoplasmic mRNAs and/or controlling the rates at which the mRNAs are translated. The question as to which of these events is dominant in the hormonal stimulation of protein synthesis has long been keenly debated; the cultured fibroblast provides an ideal system for measuring their relative importance.

It is conceivable that the translation of mRNA into protein could be governed by the availability of ribosomes. In growing cells rRNA appears to be stable (Weber, *Nature new Biol.*, **235**, 58; 1972; Abelson *et al.*, *Cell*, **1**, 161; 1974), but in non-growing cells the rRNA turns over at about 1% per h. This gives a relative rate of rRNA synthesis in growing compared with non-growing cells of about four-fold based on their relative RNA content, or about eight-fold on a per cell basis. Cells which have just been stimulated would have intermediate rates of rRNA synthesis, and this has been confirmed by labelling experiments (Bandman and Gurney, *Expl Cell. Res.*, **90**, 159; 1975; Rudland, *op. cit.*). How these relatively large differences in the rates of rRNA syn-

thesis are achieved is not yet clear. Certainly increased transcription of rRNA genes occurs (Emerson, *Nature new Biol.*, **232**, 101; 1971; Mauck and Green, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2819; 1973) but whether there is also less wastage of rRNA precursors as seen in stimulated lymphocytes (Cooper, *Nature*, **227**, 1105; 1970) is not known. But although the increased rate of ribosome synthesis in cells recently stimulated can hardly have any impact on the total ribosome content for some hours, the increased rate of protein synthesis is observed very rapidly. This must mean that some other feature of the protein synthetic machinery is altered.

Keen proponents of the applicability of the Jacob-Monod model to mammalian systems might suggest that an increase in mRNA content is responsible. In both growing and resting cells the half-life of mRNA is the same (Abelson, *op. cit.*) and corresponds to a degradation rate of 8% per h. This would mean that the relative rate of mRNA synthesis in growing and resting cells was 1.4 times their relative mRNA contents. Here there is some conflict in the literature. Using steady state labelling Johnson and his co-workers (*Cell*, **1**, 95; 1974) claim that resting cells contain two to four times as much mRNA on a per cell basis as resting cells. On the other hand, using a method of quantitating mRNA by hybridising ³H-poly(U) to the poly(A) tracts of mRNA, Rudland and his collaborators found at the most a 30% increase in growing cells (*J. molec. Biol.*, **96**, 745; 1975). The former estimate would give a relative rate of mRNA synthesis of three to six-fold on a per cell basis, the latter a 1.6 to 1.8-fold increase in growing cells. In fact, analysis of the rates of appearance in the cytoplasm of ³H-poly(A) tracts attached to mRNA gives a relative rate of 1.8-fold in stimulated cells (Bandman and Gurney, *op. cit.*), suggesting that the relative mRNA contents of growing cells may be only marginally higher than in resting cells. One unexpected result from these experiments is that the increase in mRNA synthesis seen in growing cells is not due to an increase in gene transcription but rather to an approximately two-fold increase in the efficiency with which mRNA molecules are clipped from their high molecular weight precursors in the nucleus (Johnson *et al.*, *Cell*, **4**, 69; 1975). In any event, the small increase in mRNA content in growing cells is not sufficient to account for the approximately four-fold increase in the rates of protein synthesis seen in stimulated cells, especially since the increase is so rapid.

When the cytoplasmic location of poly(A)-containing mRNA in non-

growing cells was examined, it was found that a significant amount of mRNA, variously estimated at between 37% (Bandman and Gurney, *op. cit.*) and 85% (Rudland *et al.*, *op. cit.*) was not associated with polysomes, but was in the form of slowly sedimenting ribonucleoprotein particles. On stimulation this mRNA was rapidly recruited into polysomes which are formed at the expense of free ribosomes. A number of indirect experiments show that the existence of mRNA in these particles is due to a low rate of initiation of ribosomes on mRNA. After stimulation this rate is rapidly increased (Rudland *et al.*, *op. cit.*; Bandman and Gurney, *op. cit.*). It seems that the increased rate of initiation of ribosomes on mRNA and the resulting recruitment of pre-existing untranslated mRNA is sufficient to account for the rapid increase in protein synthesis seen after serum stimulation. Later, the extra concentration of mRNA and ribosomes will help maintain the high rates of synthesis.

The conclusion reached from these results is that the substantial changes in the general rate of protein synthesis seen in stimulated cells are governed largely at the translational level rather than at the level of mRNA transcription. Gratifyingly, similar conclusions have been reached using much more indirect experiments for the action of trophic hormones *in vivo* (Korner, *Ciba Foundation Symposium*, **86**, 1970). Induction of specific proteins in animal cells however such as the induction of oralbumin by oestrogen in the Chick oviduct, may rely more on changes in the amount of specific mRNAs. □

Man against nature in the climatic stakes

from John Gribbin

LATEST indications suggest that all of man's polluting influences on climate may be acting to produce a global warming, while the natural trend extrapolated from past variations is towards a cooler regime. In the short term, the former influence may well dominate, and this suggests the need for a radical rethinking of some predictions, such as those concerned with the impact of climatic change on agriculture and world food production.

Until recently, it could be argued that some aspects of man's activity cause a warming tendency (CO₂ and the greenhouse effect) while others tend to cool the Earth (atmospheric dust) so that on balance they might be ignored. But last year several calcula-

tions were published showing that the net effect of the aerosol dust particles might also be to cause a global warming (*Nature*, **253**, 162; 1975). More recently, R. A. Reck has suggested that the present background aerosol density should be contributing a temperature increase of 0.2 K at 85°S and 0.05 K at 85°N, acting to oppose the increase in snow and ice cover in 1971 which some climatologists have linked with circulation changes associated with the Sahelian droughts of the early 1970s (*Science*, **188**, 728; 1975). The difference in the size of this effect in the two hemispheres arises because it depends on the relative albedos of the aerosols and the underlying surface, and there is of course much more land in the north.

So what of the greenhouse effect? Considering CO₂ alone, and ignoring any effects due to dust, W. S. Broecker has suggested that a pronounced warming could become apparent within a decade (*Science*, **188**, 460; 1975). There have been many calculations of the direct warming which would be caused by a specific increase in CO₂ content of the atmosphere, and although these have not always agreed with one another the differences between them are now small enough for Broecker to produce a plausible extrapolation of the exponential growth of this contribution to global temperature up to the end of this century. Where his study goes a step further than those of most workers is in combining this warming trend with an extrapolation of the 'natural' trend of temperature from the pseudo-periodic fluctuations of the ¹⁸O record in the Camp Century ice core.

The combined effect of the two processes is likely, on this model, to produce dramatic changes in the near future. The natural cooling phase since the mid-twentieth century has been opposed by the growing greenhouse effect, the net result being only a modest cooling; but according to the ice core evidence, this trend is likely to reverse within ten years, and from then until the end of this century the natural trend and the greenhouse effect will combine to produce a pronounced warming, increasing mean global temperatures by half a degree or more above the present level. According to Broecker, the greenhouse effect would then be operating so strongly that future natural cycles with the 80-year Camp Century 'period' would serve only to modulate the ever steepening rise in temperature. This, he says, "by early in the next century will have driven the mean planetary temperatures beyond the limits experienced during the last 1,000 years". In fact, that may be no bad thing, since the past millennium has been, by and large, cold and

unsuitable for the world to support anything like the population expected for the end of this century. But as Broecker stresses, the agricultural consequences of such a warming are not obvious, and we do not know how a change in mean global temperature would be likely to affect rainfall patterns in the all important grain producing regions.

Some aspects of Broecker's study are certainly speculative, but none the less stimulating in highlighting the need to consider the possible impact of such a warming, and not to be too restricted and look only at the problems posed by cooling trends. The need to avoid the stereotyped approach is also highlighted by the discovery that chlorofluorocarbons—familiar to doomwatchers as possible destroyers of the ozone layer—may also contribute directly to climatic change, again through a greenhouse effect.

These compounds are strongly absorbing at infrared frequencies, and V. Ramanathan calculates (*Science*, **190**, 50; 1975) that if the concentration of them in the atmosphere reaches two parts per thousand million there could be a corresponding increase in surface mean temperature of more than 0.25 K. This is the concentration which may be reached by the year 2000 if present levels of injection continue (Molina and Rowland, *Nature*, **249**, 810; 1974), and although smaller than the projected CO₂ influence this would be significant alone. With this effect and perhaps the aerosols adding to the CO₂ effect there seems nothing to oppose the warming influence of man's polluting activities except the natural variations of climate.

Those natural variations must dominate in the long term, and even within a hundred years of CO₂ production at the rate implied by Broecker's extrapolation there will be little prospect of further significant increases since there will be little fossil fuel left to burn. So the pronounced global warming he envisages is, even if genuine, only likely to be a short term phenomenon, assuming that plants continue to take up CO₂ from the air. Looking further ahead to the next 1,000 years (which is a short enough span in climatic terms), the overall cooling tendency of the globe is likely to continue into a full ice age, according to studies of isotope variations in cores drilled from the floor of the Antarctic sea. These studies, made by a team at Lamont-Doherty Geological Observatory, were reported to the recent Norwich conference on climate by J. Hays.

Hays' interpretation of long term climatic fluctuations from this evidence provides strong support for the Milankovitch theory that changes in the tilt and precession of the Earth affect in-

solation at critical latitudes sufficiently to cause ice ages, and suggests that while ice ages are roughly synchronous in the two hemispheres the south leads the north by a few hundred or a thousand years. Since the most recent sediments show isotope variations implying that the southern hemisphere has already begun to move towards a new ice age, the conclusion that a full global ice age is due within a millennium follows inevitably from this interpretation of the past variations. In that case, and looking at the lifetime of our civilisation rather than that of an individual, it seems we should make the most of any warm spell we do get in the coming decades, since there is a long cold spell to follow. □

Ecological carousel

from Peter D. Moore

ECOLOGISTS, like the Greeks, are seekers after ideals. Among conceptual ideals, none is less tangible, or more sought after, than that of the ecological climax. The idea of the perfectly stable, equilibrium ecosystem which has developed through a sequential series of successional stages, is a very attractive one, but even as the concept evolved in the minds of such men as Clements, it gave rise to ecological controversy. The Clementsian ideal of a climax system in equilibrium with climate was soon demonstrated to be impracticable when applied by Tansley to vegetational studies within the British Isles. Tansley insisted that stability was the essence of climax and therefore any overriding factor of the environment, be it edaphic, topographic or biotic, which limits the development of an ecosystem short of its potential climatic climax state, can be the determinant of a climax system.

The development of ecosystem theory in recent years has necessitated a restatement of the climax ideal, and this has been provided by Odum (*Science*, **164**, 262; 1969), who based his definition on the characteristic energy flow and nutrient cycling patterns to be expected from a steady-state system. In practice, however, as in the case of the Clements-Tansley debate, much depends upon the scale, both temporal and spatial, on which any system is being studied. Apart from natural calamities, which are of frequent occurrence in many ecosystems (see Loucks, *Am. Zool.*, **10**, 17; 1970), the inevitable death and collapse of climax dominants, particularly in forest ecosystems, will result in renewed succession with a frequency dependent upon the generation time of the dom-

inant. Consequently, the stable system found in the real world consists of a mosaic of microsites each of which may be in a different state of successional development or dominant maturation. The whole system may, nevertheless, approximate to the Odum ideal in terms of its energy and nutrient balance sheet.

This state of affairs is well documented in a recent study by Forcier (*Science*, **189**, 808; 1975) of the canopy tree species in a forest in New Hampshire. Within this forest, 90% of the trees belonged to one of three species, namely beech (*Fagus grandifolia*), sugar maple (*Acer saccharum*) and yellow birch (*Betula alleghaniensis*). Forcier observed 400 randomly chosen plots within this forest over a period of three years and paid particular attention to the population dynamics of the seedlings of these species. Mortality of beech seedlings was found to be high initially but declined with age, thus average life-expectancy increased. Birch, on the other hand, had a fairly constant mortality whatever the seedling age. Sugar maple was intermediate in its population dynamics. Also, beech seedlings were found to vary little in their density whilst birch seedling densities varied considerably and average density was more than five times that of beech. These data suggest that birch is behaving as an opportunist in the composition of the forest.

Association analyses between the seedlings and the canopy trees showed that sugar-maple seedlings were associated with birch canopies and beech seedlings with sugar maple canopies. Evidently the three species are related in a cyclic successional series in which disturbance (or death of a mature tree) leads to birch invasion, sugar maple fares well beneath the birch canopy, eventually replacing it, and finally beech invades and replaces sugar maple. The roughly equal abundance of the three species of tree in the canopy is indicative of the importance of this cyclic micro-succession in what appears superficially to be a stable, 'climax' forest.

Forcier calls this a 'near climax ecosystem', but this is rather misleading, since it suggests that the system falls short of the ideal. In a sense it does, for the community consists of a mosaic of sites, most of which are in different stages of the cyclic development. Nevertheless, the forest as a whole complies with many of the demands of the Odum ideal. There is no net increase in biomass, so gross ecosystem production is equalled by ecosystem respiration. Also the total input of nutrients from all sources (meteorological, geological and biological) must be equalled by total outputs. Reality fails to live up to the ideal only in that

the 'stable' system contains species which cannot regenerate efficiently beneath their own canopy and which one therefore tends to associate with successional stages instead of the climax. Rather than call this forest a 'near climax', let us be ready to modify further the climax dogma and admit that climax is a carousel. □

What drives the solar cycle?

from Nigel Weiss

IAU Symposium No. 71 on Basic Mechanisms of Solar Activity was held in Prague on August 25-29, 1975.

THE Sun is the only star that can be observed in detail and any theory of stellar structure must be tested against these observations. Unfortunately, conventional models cannot explain the low measured neutrino flux or the excitation of oscillations in the Sun: as I. W. Roxburgh (Queen Mary College, London) put it, "solar physics has forced the stellar physicist to think again." Over the past few years solar physics has developed rapidly. Observationally, it has become possible to resolve structures with scales smaller than the photosphere granulation, while theory is advancing from simple mechanisms to detailed computations.

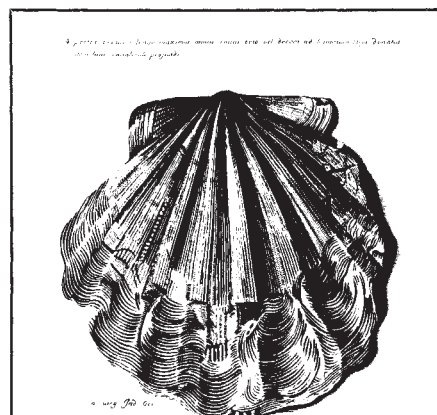
The solar cycle is apparently typical of stars with convective envelopes and

O. C. Wilson (Hale Observatories) has found similar behaviour in several late-type stars. Magnetic fields on the Sun have been observed in profuse detail, from satellites and from the ground; indeed, we have far less information about fields in the Earth's core, which is shielded by the mantle. It is generally accepted that the cycle is maintained by an oscillatory dynamo yet E. N. Parker (University of Chicago) emphasised that many features of this process are still poorly understood.

The orthodox model is a dynamo driven by a combination of differential rotation and helical motion in the outer convective zone. A dipole-like field is wound round the Sun by differential rotation and the resulting toroidal field is twisted by helical convection to produce a reversed poloidal field. Samples, particularly of the strong toroidal field, pop through the surface in active regions. This process can be represented by mean field electrodynamics: F. Krause and K.-H. Rädler (Zentralinstitut für Astrophysik, Potsdam) described the procedure of separating large scale mean fields from small scale fluctuating fields and then averaging over space or time. Turbulent convection provides an enhanced eddy diffusivity and, through helicity (the α -effect), generates the poloidal field. Turbulence also produces fluctuating fields which, in the Sun, may be 100 times greater than the mean field itself. M. Stix (Göttingen University) discussed numerical solutions of the linear dynamo equations. With carefully chosen distributions of helicity and angular velocity, these yield remarkably accurate reproductions of the actual solar cycle.

Mean field electrodynamics is not perfect, however. The growth of the field is limited by non-linear effects that are not properly incorporated in the theory. Moreover, the procedure of averaging and smoothing cannot be justified for giant convective cells and highly concentrated fields. J. O. Stenflo (Lund University) has established that more than 90% of the photospheric magnetic flux is concentrated into small regions, a few hundred kilometres across, with fields of around 1,500 gauss. Outside these regions, except in pores and sunspots, only weak, irregular fields are detected. To describe such a jagged field distribution, a more elaborate theory is needed.

Unfortunately, the pattern of motion in the deep convective zone remains unclear. R. Howard and H. Yoshimura (Hale Observatories) have accumulated Doppler measurements of the differential rotation which also provide some evidence for the existence of giant cells extending through the whole depth of the convective zone. W. J. Wagner (Sacramento Peak Observatory) showed



Reproduction of the illustration of the earliest figured and described fossil from America. From Lister's (1687) *Historiae Conchyliorum Liber III*. Lister's entire description is with each figure and not in a separate text. Say (1824) mentions this figure as being his species *Pecten jeffersonius*. (Reproduced from "*Chesapecten*, a new genus of Pectinidae (Mollusca: Bivalvia) from the Miocene and Pliocene of Eastern North America"—*Geol. Surv. Prof. Pap.* 861.

that coronal holes have an almost constant rotation rate which, with the sector structure of interplanetary fields, suggests the presence of cells elongated parallel to the rotation axis of the Sun. There is as yet no adequate theory of vigorous convection in a compressible fluid, though the interaction between convection and rotation has been studied for incompressible fluids. B. R. Durney (National Center for Atmospheric Research, Boulder, Colorado) found that the observed uniformity of the energy flux at the solar surface seemed incompatible with an angular velocity that increases inwards (as required for dynamo models). P. A. Gilman (NCAR) described numerical experiments on convection in a rotating spherical shell which led to large variations of angular velocity, though not necessarily to an equatorial acceleration. It is through such large scale computations that further theoretical progress must be made.

Turbulent diffusion is an essential feature of any solar dynamo, though there is no complete theory for the reconnection of magnetic fields on a sufficiently rapid time scale. The generally accepted view is that turbulence can bring opposing fields close enough together for dynamically driven reconnection to take place. E. R. Priest (St. Andrew's University) and A. M. Soward (Newcastle University) demonstrated that rapid reconnection can occur at rates up to the Alfvén speed. J. H. Piddington (CSIRO, Sydney) dissented from the orthodox approach. He believes that turbulence may redistribute magnetic flux but is unable to destroy it. Indeed, his magnetic fields form isolated flux ropes that control the pattern of cellular convection (instead of being concentrated by the motion). So he has revived an alternative model of the solar cycle, relying on a primordial steady poloidal field from which the alternating toroidal field is generated by some form of torsional oscillation.

Immunological tolerance

from a Correspondent

A symposium on Immunological Tolerance was held at Reinhardtsbrunn, GDR on October 14-17. It was sponsored by the International Union of Immunological Societies and the World Health Organization.

THE mechanisms of antigen-induced unresponsiveness (immunological tolerance) are still controversial: tolerance

has been ascribed either to "deletion" of relevant lymphocyte clones, or to active suppression of these clones by other lymphocytes (T suppressor cells) or by humoral "blocking factors".

The classical concept of clonal deletion in tolerance to transplantation antigens, first propounded by Sir MacFarlane Burnet and others, has withstood the test of time. Experiments by H. von Boehmer and J. Sprent (Basel Institute for Immunology) with mouse radiation chimaeras (F_1 hybrid mice reconstituted with bone marrow cells from both parents), provide clear evidence that self-tolerance in these animals is due to clonal elimination and not to suppression.

Evidence for blocking factors in the maintenance of tolerance was also presented, however. H. Wekerle (Max Planck Institut, Freiburg) has induced autoreactive lymphocytes by co-culturing lymph node cells and testicular cells from the same rat. The cytostatic activity of these cells is blocked by an unknown factor present in autologous serum, and to a lesser degree by serum from allogeneic rats. M. Embleton (Cancer Research Campaign Laboratories, Nottingham) discussed experiments showing that rats developing hepatomas first have free tumour antigen in their serum, and then antigen-antibody complexes. The latter are effective in "blocking" the activity of effector lymphocytes, and disappear from the circulation within 3 days of the excision of the tumour.

Inhibition of lymphocyte effector function by T suppressor lymphocytes was a major topic of discussion. Both K. Eichmann (University of Cologne) and Leonore Herzenberg (Stanford University) presented evidence that suppressor T cells act by inhibiting the function of helper T cells (cells which help B lymphocytes to make antibody). Data from A. Basten (University of Sydney) suggest, however, that under some conditions the target of suppression may be the antibody-forming cell precursor (B cell).

There were, nevertheless, close parallels between these three systems. Thus, suppressor cells seem to belong to a discrete T cell subpopulation—bearing Ia antigens, distinctive Ly antigens, and with a characteristic tissue distribution. Most strikingly, both Eichmann's and Herzenberg's work suggests that there are categories of helper T cells which only interact with a restricted B cell subset—those destined to produce a single allelic variant of one immunoglobulin subclass (Herzenberg) or a single idotype (Eichmann). These specialised helper cells seemed to be the targets of suppression.

The general role of suppressor cells in tolerance remains unresolved. Basten presented clear evidence that although

mice made tolerant with human gamma globulin developed suppressor cells, the animals remained tolerant long after the suppressors had disappeared. For the present one may conclude that, although suppressor cells are demonstrable in a number of tolerance models, they have not been shown to be obligatory either in the induction or maintenance of unresponsiveness. Rather, they may fulfil a more subtle homeostatic function.

One session was devoted to tolerance induced by "suiciding" specific immune responses by attaching a lethal agent to an antigen, so that the complex inactivates clones of cells bearing receptors for that antigen. This conceptually attractive approach, with obvious potential therapeutic applications, has hitherto been largely restricted to animal models using highly radiolabelled antigens. Recently, however, H. Ambrosius and his collaborators at the University of Leipzig have been testing various immunosuppressive agents, in particular 6-mercaptopurine, coupled to protein antigens. Such conjugates specifically suppress anti-protein antibody synthesis in guinea pigs. D. A. L. Davies (Searle Research Laboratories, High Wycombe) is studying the effects of a phenylalanine mustard coupled to anti-tumour antibody on the growth of a transplantable mouse lymphoma. The results are encouraging: administration of the drug or antibody alone is ineffective, giving the drug at the same time as antibody is effective, while the covalent drug-antibody conjugate is even more effective.

The success of this meeting was undoubtedly partly due to the fact that it brought together experts in various aspects of tolerance, and enabled them to find common ground for a variety of phenomena. Most importantly, however, it also provided a unique opportunity for immunologists from the East and the West to meet and exchange ideas. □



A hundred years ago

AT its last sitting the French Geographical Society broached a scheme for inducing the several French Railway Companies to place at each station a map of the vicinity, with indications of the most notable historical or economical facts connected with the district. It appears that this is the universal practice on Brazilian railway lines.

from *Nature*, 13, 77; Nov. 25, 1875

review articles

Visual experience and cortical development

H. B. Barlow*

The past twelve years have been exciting for those interested in the classical question, "How much do our visual capacities depend on innate developmental factors, how much on the moulding effects of visual experience?" Recent papers¹⁻⁴ have settled some issues, but may start new waves of controversy, and this is the occasion for writing this review.

THE oldest source of knowledge on visual development is clinical, for ophthalmologists know from their experience with amblyopia that one eye can become almost completely functionless from lack of use. This most commonly occurs when the two eyes interfere with each other, either because of strabismus (squint), or because one eye has a much greater refractive error than the other, and it can be partially prevented and reversed, at least in its early stages, by patching the good eye, thus forcing the use of the "lazy" eye that is threatening to become amblyopic. In 1963, Wiesel and Hubel⁵ reported similar effects produced in cats by interfering with the normal binocular use of their eyes in the first month or two after they opened, and they discovered that the underlying change occurs in the cells of the primary visual cortex (area 17). In normal animals a high proportion of these neurones receive connections from both eyes⁶, but if one eye is covered soon after birth, it makes connections with very few cortical neurones, almost all of which are then found to be connected solely to the eye which has been in continual use⁷. Furthermore, if a kitten is made strabismic by sectioning an eye muscle, or if joint usage of the eyes is prevented by covering each of them alternately, their neurones are found to receive input from one eye or the other, but very few receive input from both⁷.

In their experiments Hubel and Wiesel found that covering both eyes had comparatively little effect on the cells of the cortex, suggesting a competitive element in the causation of the changes from monocular deprivation⁸. This was confirmed by Sherman, Guillery, Kaas and Sanderson⁹ who made retinal lesions in one eye and closed the other. They found reduced effects of deprivation in the parts of the visual field that had no competing representation in the open eye. Hubel and Wiesel¹⁰ found cells in the cortex of very young kittens, before the age of eye opening, that were connected to both eyes and appeared normal in other properties, and they reproduced many of these results in monkeys¹¹. The apparent normality of cells in very young visually inexperienced animals, and also in binocularly deprived ones, led them to believe that the functional pattern of connections that underlies normal vision are laid down by genetically determined developmental processes. These can be interfered with or disrupted by abnormal visual experience in the "sensitive period" of the first few months of life¹², but they did not think that normal visual

experience had any constructive effect in setting up the neural apparatus in the primary visual cortex.

These very important experiments with their suggested answer to the ancient dispute about the role of experience have naturally aroused great interest, and some controversy has arisen about both the facts and their interpretation. By now most of the facts discovered by Hubel and Wiesel have been confirmed by others, but there have been further advances that will be considered under four headings: ocular dominance and binocularity; specificity without experience; disparity selectivity; and the degree of modifiability of cortical neurones.

Ocular dominance and binocularity

The effects on the monocular and binocular connections of cortical neurones have proved reliably repeatable, but interesting additional knowledge has been gained by the technique of reversed suture¹³⁻¹⁵, in which the originally closed eye is opened and the originally open eye is closed. It seems that an eye that has become disconnected can be persuaded to reconnect by renewed visual experience, provided that this occurs during the "sensitive period" and provided that the newly opened eye does not have to compete with an already connected eye that is in continued use. Thus experience can certainly have a reconstructive effect, and it is particularly interesting that the reconstructed connections are different in several ways from the previous connections that had been disrupted by the period of closure. The most striking evidence for this is provided by neurones that are in the process of being taken over by the newly opened eye but can still be driven by the other, previously open, eye. Some of these are quite unlike any cells found in normal cortex in having widely divergent preferred orientations for the two eyes, the difference sometimes being more than 70°, which is never found in neurones of normal cats and kittens. More information about this is obtained by examining the properties of the series of neurones encountered while advancing an electrode through the cortex, for the newly formed connections seem to develop a sequential pattern of orientational selectivity that is similar to the old pattern, but independent and often out of step with it¹⁶.

The effects of deprivation are certainly easier to show than are those of experience, and a nice example of this was given by Olson and Freeman¹⁷. They found that 2-5 d of monocular vision had a profound effect on the ocular dominance histogram, but unexpectedly, it made no dif-

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ference whether the animals had been reared in total darkness or in ordinary light before the monocular experience. For the deprived eye, several weeks of ordinary vision were undone in a few days of deprivation. Following reversed suture, however, one can see that experience encourages reconstruction, and one must suspect that normal joint usage of the two eyes may have some positive effect in establishing the normal orderly matching of the connections from the two eyes.

Specificity without visual experience

The properties of neurones in the adult primary visual cortex vary greatly, but it can be said of the vast majority that they selectively respond to only a very small proportion of the vast gamut of possible visual stimuli⁶. They are selective according to (1) the position of the stimulus, (2) whether lighter or darker than the background, (3) its direction and velocity of movement, (4) its size and orientation, and (5) the disparity or relative positioning of the image in the two eyes^{18,19}. All are agreed that neurones with selectivity for (1), (2) and (3) occur in very young kittens without visual experience. Hubel and Wiesel also said there were many cells with the normal selectivity for the orientation of an extended object, as well as normal binocular connections, and it was on this basis that they concluded that all selectivity was innately determined. Barlow and Pettigrew²⁰ and Pettigrew²¹ found clear-cut selectivity for the direction of movement of a stimulus in animals with no visual experience, and thought that the apparent orientational selectivity could be attributed to this directional selectivity. This seems to be wrong, for although many of the cells in very young animals are abnormal in their selective properties, there are about 25% (refs 2, 4) with the orientational properties of adult simple cells.

With regard to older, totally deprived, kittens Hubel and Wiesel again reported many normally selective cells, whereas Barlow and Pettigrew found a remarkable lack of them, and in this respect later work has supported them^{2,4,22}. Even without the evidence from older kittens, however, it is now clear that all the types of selectivity of area 17 neurones, except that for binocular disparity, occur with no visual experience. The question whether they have as high a degree of selectivity as adults is still open, and there does seem to be a possible role of experience in "tuning up" their selectivities. This is a point of some interest because of the slow attainment of the high acuity characteristic of adults (*vide infra*).

Disparity selectivity

Most studies have followed Hubel and Wiesel in representing the binocular-monocular properties of cells in the visual cortex by "ocular dominance histograms". Cells are classified into seven categories varying from those excitable only through the contralateral eye (group I), through those excitable equally well by either eye (group IV), to those excitable only by the ipsilateral eye (group VII). Such histograms tell one the relative strengths of the excitatory inputs from each eye, but they tell one nothing about the way the inputs from the two eyes interact. This is important, not only because of possible facilitation, summation, or occlusion, but also because the input from an eye that does not excite a neurone can nevertheless inhibit it²³. Thus the fact that ocular dominance histograms of very young kittens resemble those of normal adults does not mean that these animals handle binocular inputs normally. Pettigrew²¹ studied this aspect of the problem in developing cortex and found a marked increase in the degree of selectivity for disparity with age and visual experience. Binocular interactions of young or inexperienced neurones occurred, but were much less precise and lacked the inhibitory component prominent in older and experienced

neurones. The implication is that very young and totally deprived animals have normal ocular dominance histograms, but the critical inhibitory interactions of adults develop only with experience. This deserves repetition, especially in view of the contradictions about orientational selectivity, for it is again strong evidence for a constructive role of experience in forming the connections which combine the inputs from the two eyes.

Modifiability of other properties

A controversial problem is whether properties of the population of cortical neurones other than binocular connectivity can be affected by experience. Hirsch and Spinelli^{24,25} and Blakemore and Cooper²⁶ reported dramatic results induced by exposing kittens to striped patterns, and Shlaer²⁷ produced some evidence that a prism in front of one eye deviates the receptive field positions of that eye. These results were extended in later reports^{28,29} and since then modifications have been described as a result of rearing animals in stroboscopic light^{30,31}, in an environment of moving stripes³²⁻³⁵ and in a visual environment of dots without any stripes or lines^{36,37}. Some of these treatments are claimed not just to modify the proportions of neurones of different types, but also to produce neurones with properties totally unlike any that are found in normal cortex. For instance Hirsch and Spinelli²⁵ reported units that seemed to have a receptive field exactly matching several bars of the grating the eye had been exposed to, and a close matching of receptive field to stimulus also seems to occur when kittens are raised in an environment of dots without lines^{36,37}. The cortical units reported for these kittens would certainly be most unusual in normally reared animals.

Another example of abnormal units was described by Pettigrew, Olson and Hirsch³⁸ in the cortex of cats reared with one eye viewing vertical, the other horizontal, stripes, for they found units, like those described by Blakemore and Van Sluyters¹⁴ following reversed suture, that had widely divergent preferred orientations in the two eyes. Again, when one eye is rotated surgically but not closed in young kittens³⁹, the position of the receptive fields at different points in the cortex stay unchanged relative to the retina. The orientations are not however normal: after correction for rotation the preferred orientations in the two eyes may be widely divergent in the few remaining binocular neurones. Once more we have evidence that co-ordinated binocular stimulation is needed to obtain coordinated development of the appropriate selectivities.

Behavioural consequences of selective visual diets have also been described^{40,41}, though these are rather subtle and hard to observe compared with the effects of monocular deprivation^{42,43}. There have been no reports of animals or humans with supernormal visual capacities following abnormal early visual experience, though this might be expected to arise according to some views. Some failures to obtain modification have been reported but hitherto these could be explained by differences of species, as when rabbits were used^{44,45}, or procedure, as when Maffei and Fiorentini¹⁶ used vertical stripes of rather limited vertical extent and exactly regular periodicity.

At this point it seemed that, although most forms of selectivity were detectable in inexperienced cortex, they were also modifiable by subsequent visual experience. The report from Stryker and Sherk¹ however, has reopened the whole issue, for they made a careful and thorough attempt to reproduce the stripe-rearing experiment, following the procedures of Blakemore and Cooper, but they were unable to obtain significant results. The main points of difference were (1) an improved method of selecting the cells to be recorded from, aimed at obtaining more representative samples; (2) the use of a computer to control the stimulus and record the results, instead of hand-moved targets and

subjective impressions; (3) ensuring that the experiment was done "blind", without the experimenters knowing the rearing conditions. Many people are likely to conclude that experimenter bias, reliance on subjective impressions, and an effectively very small sample of cells explain the dramatic findings of the previous experimenters, though it is not clear whether Stryker and Sherk hold this opinion. Against this conclusion is a brief study by Pettigrew, Olson and Hirsch³⁸ that was done blind, with computer control, and with positive results. There are further results, as yet unpublished, from Pettigrew's laboratory, and some of the papers already quoted contain incidental confirmation of the stripe-rearing effect (see also the note added in proof to this article). Obviously one must consider Maffei's and Fiorentini's⁴⁶ negative results again, but it seems to me unlikely that so many reasonably careful people should have been fooled so much of the time, and the clinical evidence to be described below establishes that orientational properties can be modified. It is therefore worth considering other possible explanations for the negative results.

The condition of the kitten when viewing the stripes is the natural point to probe. Was it alert? How much time did it spend looking up or down, and thus deviating the lines from their intended horizontal or vertical? What else did it see? Was it hungry, interested, rewarded? All these factors are hard to control, but may be expected to influence the results if the modification is anything like those occurring during normal learning. As a possible physiological explanation it is worth pointing out that Otten and Thoenen⁴⁷, studying the effect of preganglionic stimulation in inducing enzyme formation in sympathetic ganglia, had at one point great difficulty in getting reproducible results. This was traced to the glucocorticoid level in the blood at the time of induction: if the experiment was done in the morning, when the level was low, induction failed. Perhaps Stryker's and Sherk's cortical neurones ignored the stripes for the apparently irrelevant reason that they were exposed at the wrong time of day. Another possibility is that the sensitive period for orientational lability is earlier than that for ocular dominance, as Daw and Wyatt suggest for modifiability of directional selectivity³⁵. There is also some evidence that dominance changes are slower than changes of orientation^{29,48}. At all events the matter is unlikely to be accepted as closed and one must hope for more conclusive experiments in the future.

Evidence from psychophysics

These contradictory results go some way towards justifying the remark of one of the founders of cortical neurophysiology: "Recording from single units gives interesting results, but they are usually wrong". He was probably referring to other people's results, but he generously went on to say, "Visual psychophysics is dull but usually right". Even though psychophysicists do not necessarily share this confidence, it is worth trying to relate the neurophysiology to measurable psychophysical function, for there is new evidence that bears on the question of cortical modifiability. This actually lends quite powerful support to the single-unit results which have been called into question.

The main factor that limits the resolution of the adult visual system, as measured on straightforward tasks such as the visibility of a grating, is the finite size and separation of the elements of the retina. In the fovea of man this limit occurs at about 50 cycles per degree, and is set by the spacing of the receptor cells⁴⁹. Away from the fovea, the minimum resolvable angle rises with eccentricity at the rate of nearly 0.5 min per degree, so that at 10° eccentricity only about 10 cycles per degree can be resolved. The limit here is probably also set by the retinal grain, in this case traceable not to receptors but to the separation of the ganglion cells that transmit details of the image centrally.

In the cat it seems to be the size of the ganglion cells' receptive fields and their separation that limit acuity, even in the area centralis⁵⁰⁻⁵².

Clearly, if the fibres connecting eye to brain made very diffuse or scrambled connections one would not be able to resolve the image to a dimension set by the separation of their pick-up zones in the retinal image. These fibres must, therefore, make connections in the adult that are in some sense appropriate and orderly.

It is interesting that, for some tasks, the full resolution available from the retinal mosaic seems not to be used. Andrews, Webb and Miller⁵³ investigated the accuracy with which length comparisons could be made when the markers to indicate the lengths lay up to 5° from the fovea. After allowances have been made for eccentricity, the grain size that would be sufficient to enable this task to be performed was about 10 times larger, measured linearly, than that required for grating resolution. They made the interesting suggestion that this coarser mosaic is determined, not in the retina, but in the primary visual cortex, where it could correspond to the dimension, projected into the visual field, of the repeating subunit called a "hypercolumn" by Hubel and Wiesel^{54,55}. This is the area of cortex, about 1 or 2 mm square, within which a complete cycle of ocular dominance is contained when moving across the cortex in one direction, and a complete cycle of orientational selectivity when moving in the orthogonal direction. It is thus a unit cell containing all orientation selectivities and all degrees of ocular dominance. Although the receptive fields of successive units isolated on moving an electrode across a hypercolumn show an average tendency to move across the visual field in the manner predicted from the overall mapping, an exact progression is not found^{55,56}; the units are scattered around the expected positions in an apparently random manner, so it seems that the topographical orderliness for retinal position has been replaced by orderliness for orientational selectivity and ocular dominance. On the hypothesis being advanced, the output from a hypercolumn carries the information as to which hypercolumn is involved, but no information about position within it, and the consequent discarding of information causes the loss of resolution for the type of task Andrews *et al.*⁵³ studied.

Another task that suggests massive reduction of positional information is the counting of narrowly spaced lines that has been studied recently by Atkinson *et al.*⁵⁷. They found that subjects started to make serious errors in counting even small numbers of lines when these are crowded close together: possibly the output from a hypercolumn can only signal "one" or "many", and for exact counting the lines in the target must fall on separate hypercolumns.

Thus, some tasks seem to be limited by the retinal grain, others by a coarser grain that may correspond to the hypercolumnar structure of the primary visual cortex. There are yet other tasks which at first seem to demand an even greater degree of precision than grating acuity. These are tasks like detecting the displacement of a vernier, the curvature of a line, or comparing the disparity of a pair of lines, and their common feature is that they require the detection of the relative positions of features in the stimulus that lie close to each other in the visual field. Since they are close, they could be within the area of visual field covered by one hypercolumn, and this type of information about relative position is not discarded, but jealously preserved. The precision with which these tasks can be performed is extremely high, of the order of a few seconds of arc, but Andrews, Butcher and Buckley⁵⁸ show that this is no higher than the accuracy expected from efficient use of the information in an image limited by the retinal grain. The improvement results from averaging, and simply corresponds to the fact that a mean is more precise than the individual measure-

ments on which it is based. Thus there is no mystery about how the information required for the task gets to the brain, but a highly precise and orderly arrangement does seem to be required in order to extract it, and this is presumably one of the tasks of the hypercolumns in the primary visual cortex.

To summarise, then, psychophysics suggests there must be precisely ordered connections from eye to brain to preserve details down to the size of the retinal mosaic, and an even more precisely ordered arrangement within V1 for extracting the relative mean positions of features present in the image. But there is nothing impossible or implausible in these requirements, for hypercolumns are 1 or 2 mm across and contain some 10^5 cells, which allows plenty of scope for the operations demanded. The next step could be a reduction of positional information in the visual message brought about by retaining information about local pattern or relative position, but discarding absolute positional information, thus simplifying the picture by coarsening the mosaic to that of the hypercolumnar structure of V1.

Precise, orderly, and appropriate functional neurone-neurone connections are the essence of these operations. If they are made once and for all and are not modified or varied in potency by visual experience one would have a visual system whose performance depended exclusively upon its innate connectivity, a model sometimes referred to as the "hard-wired" cortex. A hardline advocate of hardwiring will explain effects of experience exclusively in terms of disruption of innate connections, but arguments can be made on general grounds for a more constructive role. Platt³⁹ for instance held that a precision of a few seconds of arc could not possibly be laid down under genetic instruction, and would be hopelessly inflexible even if it could be. Andrews⁶⁰ argues that experience must be important in determining the metrical properties of perception, and Blakemore and Van Sluyters² suggest that experience ensures the alignment of the contributions from each eye to binocular receptive fields. Although these ideas are interesting they are not by themselves fully convincing. A teleological argument from the very existence of the sensitive period carries some weight, for it really is hard to believe in the survival value of a period of pure disruptibility, whereas a period of refinement and adjustment by experience would make good sense. At the moment there are no hard facts establishing such a role, but new evidence shows where the case for hardwiring is incomplete, and hints where visual experience may have its effects. Furthermore, recent experiments on the transfer of after effects support some of the concepts relating to cortical neurophysiology and psychophysics that have been discussed in this article.

Development of visual acuity

The neurophysiological argument for an innately connected cortex rests on the presence of selectively sensitive neurones in young, visually inexperienced kittens and monkeys. One could rule out any role of experience if such animals had full adult acuity, but this is far from the case. Acuity develops rather slowly in kittens^{61,62}, and does not reach its full adult value until quite late in the sensitive period. This could be a matter of removing other impediments to resolution, and as in all behavioural experiments one cannot be sure that the kitten is making full use of his sensory mechanisms, but the behavioural result is here supplemented by the study of cortical evoked potentials⁶¹. In humans too it seems that acuity is poor in young infants^{63,64} and reaches a value comparable with that of the adult long after the system seems to be optically efficient.

One would like to know more about the effects of early binocular deprivation on visual capacities after sight is restored, for clearly if full acuity can ever be found in an

animal that has been totally vision-deprived up to the moment of testing, this would rule out any important role for experience in performing resolution tasks. Clinical evidence on this is not very conclusive⁶⁵⁻⁶⁷ and results on animals are not yet very extensive^{13,61,62,67} but it does not seem that full visual capacities ever mature without considerable experience, and they often fail to do so if the experience occurs after infancy. If full performance cannot be achieved without experience, the implication is that normal experience aids cortical development and does not merely fail to hinder it.

Meridional amblyopia

The starting point of this whole enquiry was the fact that an eye can become blind when it is in competition with the other, functionally superior, eye, and the discovery that a similar condition occurs in cats and results from changes in the connections of cells in the primary visual cortex. It has long been recognised clinically that individuals with severe, long standing, astigmatism often fail to achieve good resolution in the affected meridian, even with the best correction of the optical condition. Testing the acuity of such subjects by generating interference patterns directly on their retinæ has ruled out the possibility of a residual optical defect, and has now shown that this "meridional amblyopia", as it is called, is really of neurological origin⁶⁸. Kittens reared wearing goggles containing cylindrical lenses have been shown to lack cortical neurones tuned to the blurred meridian, thus completing the cycle of evidence⁷⁰.

In spite of the difficulties in repeating the original stripe-rearing experiments, this clinical condition seems to establish beyond any reasonable doubt that the orientational properties of cortical neurones can be influenced by experience, though it does not of course establish that the changes are as described in the original neurophysiological experiments.

Stereo blindness

Strong neurophysiological evidence for a positive role of visual experience in setting up the visual cortex was given by Pettigrew's finding that cells narrowly selective for a particular disparity are not found in kittens that have had no binocular visual experience. The high incidence of anomalies of stereoscopic vision is therefore intriguing. Richards⁷¹ estimates that 14% of the population lack the full ability to use binocular disparities to judge depths, and he reports interesting evidence that the stereo blind may fall into at least two classes—those who cannot use the convergent disparities of objects closer than the fixation point, and those who cannot use the divergent disparities of objects lying further away than the fixation point. Now phorias, or tendencies to strabismus, are common in infancy, and could cause deprivation of binocular stimulation. If the phoria is of the convergent variety, objects at the attempted fixation distance will have divergent disparities, but the system will tend to be starved of convergent disparities. Similarly those with a divergent phoria will be exposed to convergent disparities but will tend to be deprived of exposure to divergent disparities. The two forms of stereo blindness could thus be other examples of deprivation amblyopia.

Recent clinical evidence suggests that a week of monocular deprivation in infancy can lead to unilateral amblyopia in later life^{72,73}, so the sensitivity to deprivation is certainly very high in humans. The more subtle role of phorias in causing stereo blindness seems to be another potentially fertile field for correlating neurophysiological and clinical results.

Transfer of after effects

The view that proper stereopsis depends upon experience is supported by recent experiments comparing the transfer

of after effects in normal adults and those who have seriously impaired stereoscopic vision, but have good monocular vision in both eyes, and no diplopia. From the cortical neurophysiology one might hazard a guess that they do not have disparity selective cells in area 17, and probably possess a population of neurones like that found after strabismus or alternating occlusion in kittens: that is, many cells monocularly dominated from each eye, but very few that receive binocular connections. Now there are a number of illusions such as motion after effects, tilt after effects, and distortions in the appearance and threshold modulation of gratings, that are commonly attributed to fatigue of cortical neurones; Maffei, Fiorentini, and Bisti⁷⁴ have recently shown that such fatigue does occur. Furthermore, such illusions normally transfer from one eye to the other, so that if the fatiguing stimulus is given to one eye, the illusion can be detected when that eye is closed and the other eye opened. Now if stereo-blind individuals lack binocular neurones, such transfer should be impossible, and that is precisely the result that has been found⁷⁵⁻⁷⁸. In an ingenious application of this finding Hohman and Creutzfeldt⁷⁹ have concluded that the sensitive or labile period for development of binocular neurones in humans extends to the third year.

It is interesting that Ganz, Hirsch and Tieman⁸⁰ found normal interocular transfer of learned pattern discrimination in cats reared with alternating occlusion of the two eyes and therefore presumably possessing very few binocular cells in their primary visual cortex. This result does not conflict with the absence of transfer of after effects in such a cortex, because the tasks performed are so different. The persistence of transfer of learned discriminations presumably means that they were performed at a level where information as to which eye provided the image had been discarded.

Conclusions

The neurophysiological experiments that have helped so much in understanding the normal and abnormal development of the visual cortex are difficult and arduous to perform. The preparation involves several hours of surgery, and this is followed by a prolonged period of recording during which the animal has to be maintained lightly anaesthetised and in excellent condition. Worthwhile results are rarely obtained in less than 16 h, and recording often continues round the clock for 48 h or more. On top of this there are the frustrations and difficulties of maintaining cats in colonies that are notoriously liable to obliteration by epidemics. In view of this it is not the controversy but the agreement with Hubel and Wiesel's results that is surprising, and we owe to them most of our knowledge of the visual cortex as a precisely arranged structure laid down before visual experience, and thus innate. A good deal has, however, been added to their picture, and this shows it is not really established that all is innate. First, neither an inexperienced animal, nor an inexperienced neurone, has been shown to have the normal powers of resolution, and selectivity for disparity especially seems to require experience. Second, there is the evidence that experience after monocular deprivation causes the construction of a differently organised cortex. Third, Cragg³ has recently published evidence showing that less than 1% of synapses in primary visual cortex develop before eye-opening, and hence there is, if Hubel and Wiesel are correct, an embarrassing difficulty in explaining what the other 99% do! But this merely underlines the disquieting fact that we do not fully understand what part area 17 plays in vision—itsself another reason for caution in making pronouncements upon the relative importance of innate and experiential factors in creating the intricate mechanisms that underlie our visual capacities.

There is so much to be explained in the formation and adjustment of the connections between the 10^9 or so cells of our visual cortex that it is perhaps time to abandon the idea that nature and nurture are rivals at this task. That is too simple a view; instead one should suspect that they work in collusion, and seek how and where this may occur. Does the nervous activity from experience influence the survival and effectiveness of excitatory synapses? Does it exert its effect on the pattern of connections of an intrinsic inhibitory system that is established after the innate excitatory one? Or is there an interaction between nervous activity and the development process itself, as seems to occur in goldfish tectum⁸¹? The evidence reviewed here suggests that some of the answers are still waiting to be found in the mechanisms of the primary visual cortex that analyse details of the image and combine the information from the two eyes.

Note added in proof: Three new twists must now be added to the story on the effects of selective exposure. First, Hirsch and Leventhal⁸² report more experiments in which kittens were reared wearing goggles of the type used in the original experiments of Hirsch and Spinelli^{24,25}. In their new experiments the eyes viewed stripes within the goggles that were either left oblique for one eye and right oblique for the other, or horizontal for one eye and vertical for the other as in the original experiment. For the latter condition they confirmed, using more conventional neurophysiological techniques, that cells selective for horizontal orientations were almost all connected only to the eye that had viewed horizontal stripes, and similarly for the vertically orientated cells. There were a few cells which broke this rule by responding to verticals through the horizontally exposed eye and vice versa, but there were no cells at all that responded to oblique lines. The animals reared viewing obliques had many obliquely sensitive cells connected to the appropriate eye but also had many cells which responded to horizontal or vertical stripes through each eye. They conclude that genetic factors are sufficient to develop horizontal and vertical orientational selectivity, but that experience is required for the obliques.

The second twist is a note added to Stryker's and Sherk's paper¹ confirming these results for kittens reared with the horizontal/vertical goggles, using their more precise quantitative techniques. It is not yet known whether the differences between the results using goggles, and those from Blakemore's and Cooper's cylinders, is due to more effective control of the visual experience, or whether the conflicting experience of the two eyes in the goggles is the critical factor.

Finally, Grobstein and Chow⁸³ have reviewed their own work on the development of cortical specificity in the rabbit, and have also covered much of the material in this article. Their general conclusion is that individual sensory experience is a creative factor in establishing the functional organisation of the mammalian visual system, though they believe it affects the rate of development more than the end state reached.

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articles

New evidence regarding the Quaternary geology, archaeology and hominids of Chesowanja, Kenya

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Deposits yielding australopithecines, other fossil mammals and an industry recalling the Oldowan are succeeded by an Acheulian assemblage, overlain by flood plain silts with abundant spreads of later artifacts. The structure is dominated by an asymmetric anticline. Palaeoenvironments are reconstructed and the nature of the raw materials and of the tool makers themselves are briefly discussed.

CHESOWANJA is a Pleistocene fossil and artifact locality in the Northern Rift Valley of Kenya, about 1.5 km west of the track between Mukutan and Tangulbei, at 36° 12' E, 0° 39' N, and 8 km WNW of the point at which the Mukutan river gorge cuts through the Laikipia Escarpment. A diverse fauna includes the partial cranium of a robust australopithecine^{1,2}.

We report here results from more detailed geological mapping in 1973 and 1974. A remarkable sequence of artifact-bearing levels has been discovered together with a further hominid. The original geological interpretation has been shown to be in error, necessitating re-assessments of the local stratigraphic relationships, the palaeoenvironmental setting and the age of the sequence.

The area is important because the succession of rich artifact

assemblages, from a relatively small and well defined region, spans a period probably well in excess of a million years. The associated geology and fossil fauna enable palaeoenvironmental contexts to be inferred for the various phases of hominid activity.

Geological succession

The local sequence of strata (Fig. 1) comprises four lithological units: the Chemoigut Formation, Chesowanja Formation, Karau Formation and Mukutan Beds. Formal descriptions of these and of the geology of the surrounding area will be published elsewhere.

The Chemoigut Formation consists of a sequence of silts and clays with intercalated horizons of coarse tuffaceous and pumiceous sandstone and fine conglomerate. There are calcareous bands and concretions, some of which are of algal origin, and thin flaggy and nodular calcareous horizons, several containing abundant root casts. There is also evidence of channelling. The observed thickness of the unit is ~ 25–30 m. The succession overlies red clays containing nodular calcareous, which elsewhere rest on very weathered ankaramitic basalts.

Artifacts have been found eroded out at a number of different sites from at least five horizons. Australopithecine remains come from the two highest levels. The fauna is listed in Table 1.

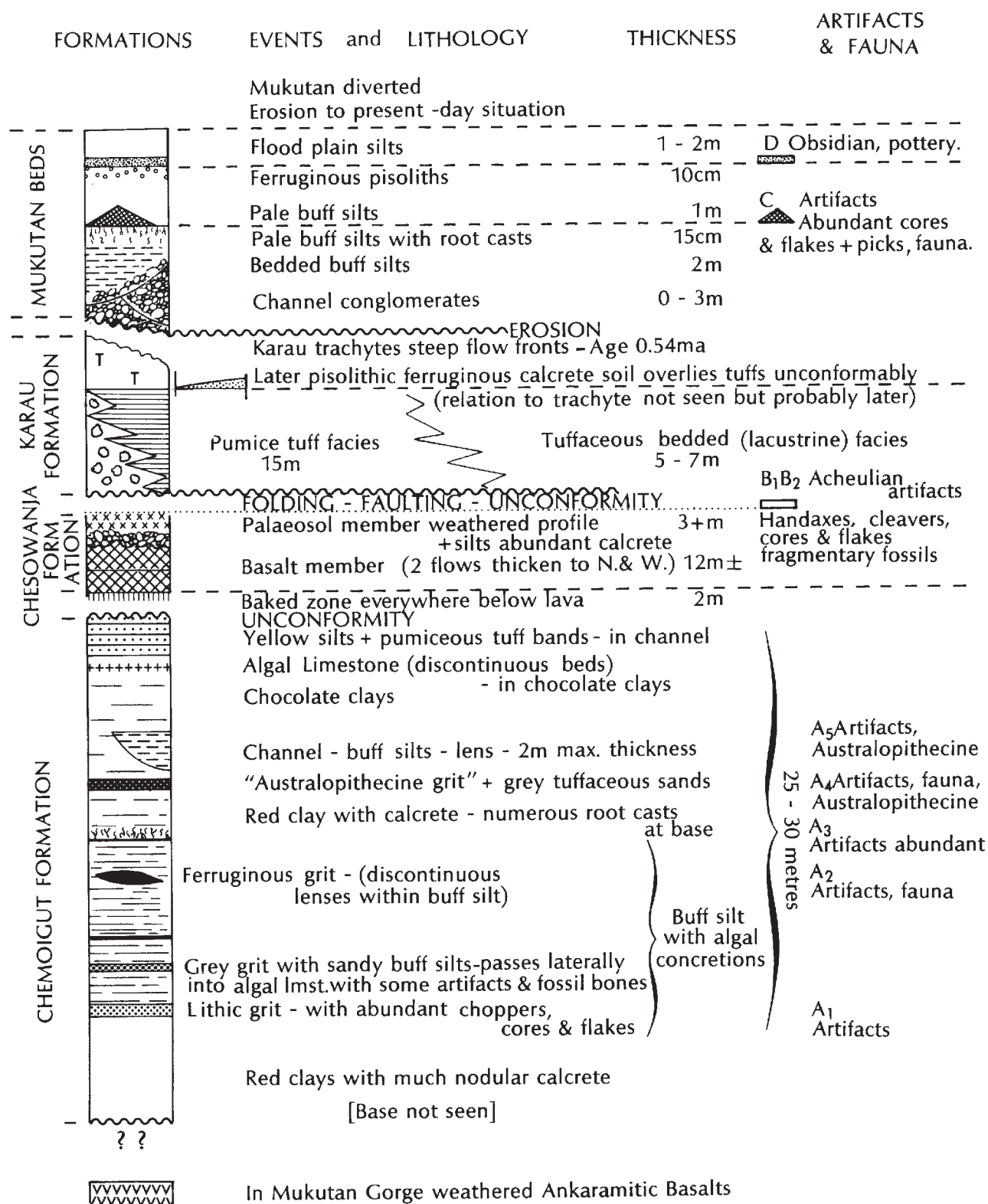


Fig. 1 Sequence of strata exposed in the Chesowanja area.

The Chesowanja Formation includes a basalt member composed of two flows, the lower of which is more fissile, and has baked and reddened the upper 2m of the underlying Chemoigut Formation. It varies from 5 to 10m in thickness. The upper flow is about 6 m thick. The surface of the upper flow is deeply weathered and large core stones of basalt occur at the base of a palaeosol complex 3 metre thick (palaeosol member, Fig. 1)

containing abundant calcrete concretions. An assemblage of Acheulian artifacts with a sparse but persistent accompaniment of fragmentary fossil material occurs at the upper surface of the formation.

In the west, the lower sediments of the Karau Formation are coarse pumice tuffs about 15 m thick, which lie unconformably over the Chesowanja Formation. The pumice tuffs can be

equated with 5 to 7 m of fine tuffaceous, well bedded sediments in the east that show graded and current bedding. These seem to represent a penecontemporaneous lacustrine facies of the pumice tuffs. The upper member is the Karau trachyte which exhibits steep flow fronts to the west of the main fossil and artifact localities.

The Karau Formation lies under local unconformities in the vicinity of the artifact and faunal areas; elsewhere it lies under a thin but persistent weathered horizon which has a patchy distribution and is characterised by ferruginous pisoliths. (see Fig. 1).

In the eastern part of the area the Karau formation is overlain by Mukutan Beds, a laterally extensive succession of channel conglomerates and silts approximately 8 m thick. The best exposures are in the Losokweta river valley and along the sides of the abandoned channel of the Mukutan River. Artifacts and fauna occur abundantly along this former course of the Mukutan.

Geometry of the units

The local relationship between the lithological units is shown in Fig. 2. The Chemoigut and Chesowanja Formations are folded into an asymmetric anticline, the axis of which trends north-south. The inclination of the beds on the western limb is about 11°. On the east they are inclined more steeply, and dips in excess of 60° are typical. The characteristically spheroidal weathered top of the upper basalt flow has been seen at only two points.

The Chemoigut sediments are exposed within the core of the anticlinal structure in three windows, each approximately 200 m across from east to west. The three windows have a total outcrop length of about 1 km from north to south along the main axis of the anticline.

Horizontal strata of the Karau Formation rest unconformably upon the flanks of the anticline indicating post-tectonic deposition. The absence of *in situ* material of this formation resting on Chemoigut sediments suggests that the Karau Formation was deposited before the fold structure in the basalts was breached. The upstanding form of the anticline at that time may account for the differing facies of the rocks on the

east and west of the feature assigned to the tuffaceous member of the Karau Formation.

The more recent Mukutan Beds crop out patchily over an extensive area to the east of the Losokweta River, where they lie under the westerly sloping pediment and wash plain at the foot of the Laikipia Escarpment.

Another hominid

The partial cranium of a robust australopithecine (KNM-CH-1) reported previously², came from the unit now called the grey tuffaceous grit within the Chemoigut Formation. More hominid fragments were found in 1973, belonging to another individual (KNM-CH-302). They came from within chocolate clays of the same formation (see Fig. 1) some 3 m higher in the sequence than the original cranial fragment. Although the fragments are surface finds, the local topographic situation (an isolated clay spur yielding fresh artifacts) suggests that they were not far removed from their original position.

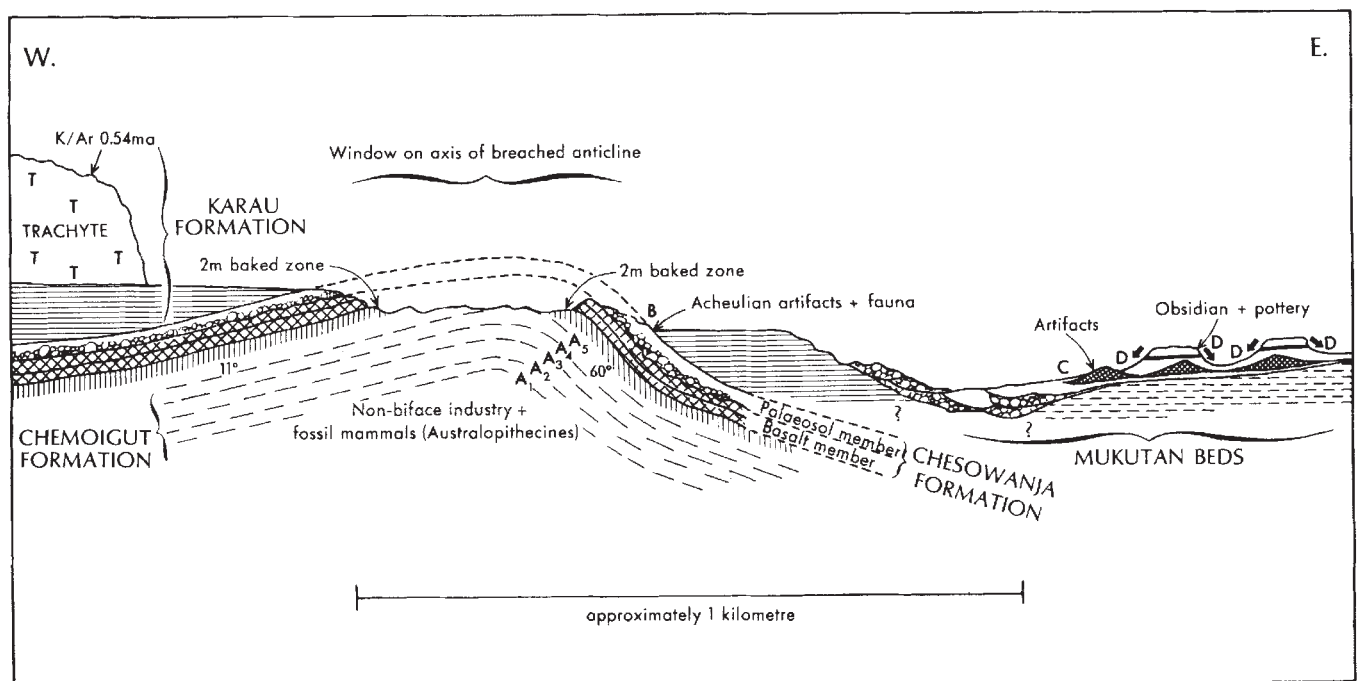
The specimens consist of a number of fragments of two molar teeth, probably of the same individual. One is a fragmentary right M¹ or M² crown showing features typical of the dentition of robust australopithecines. From the material available it is impossible to ascertain the exact dimensions of the tooth.

Archaeology

Archaeological material comes from at least eight levels within the vertical sequence of some 50 m of sediment and lava already described. All the occurrences are within about 1 km of each other. From their stratigraphic setting the artifacts can be assigned to four age groups, indicated as A₁, A₂, and so on, B, C, and D (Figs 1 and 2).

Group A. These assemblages of cores, chopping tools and flakes without bifaces and cleavers are confined to the Chemoigut Formation. They occur on at least five levels, including those from which the hominid specimens and other mammalian fossils also come. The five artifact-bearing levels have at least ten rich sites from which associations of artifacts are being eroded. No excavations have yet been undertaken but small samples from four of the sites have been collected. The 220 pieces include: 17 choppers; 8 polyhedrons; 4 discoids; 3

Fig. 2 Diagrammatic section across the Chesowanja area showing relationships between lithological units (not to scale). (We thank Dr Mary Leakey for the comment that the 'non-biface industry' may prove to be Developed Oldowan.)



protobifaces; 6 scrapers; 5 sundry tools; and 177 samples of waste, debitage, flakes, and so on. In spite of a detailed search no obvious hand axes or cleavers were found within the Chemoigut Formation windows, although they are abundant in group B.

Group B. An Acheulian assemblage with bifaces, cleavers, cores and flakes, comes from the surface of the palaeosol member. The latter is a complex weathering profile developed on the upper flow of the basalt member of the Chesowanja Formation, on the eastern limb of the anticline. At another locality on the same horizon small flakes and cores are dominant. A rather different, younger assemblage of artifacts occurs in association with the weathered horizon containing ferruginous pisoliths.

Group C. A third group of artifacts comprises mainly large cores with piles and spreads of associated flakes, together with occasional bifaces showing an economy of flaking technique, double ended picks, and beaked implements recalling the type 'Sangoan' in morphology^{3,4}. Very large local accumulations of this material are found at numerous tool sites along the dry river bed marking the former course of the Mukutan. They are being re-excavated from the pale buff silts comprising the former flood plain of the Mukutan by modern stream action and sheet erosion.

Group D. Obsidian flakes and pottery occur intermingled with debris of group C. They seem to be eroding out from an horizon characterised by the presence of ferruginous pisoliths, stratigraphically a little above the level of assemblage C within the Mukutan Beds.

More recently, the river Mukutan has been diverted for irrigation purposes. Wood from a burnt stump of a large tree along the abandoned channel has yielded a radiocarbon date of 230 ± 110 yr b.p. (unpublished, Birmingham University Radiocarbon Laboratory Date BIRM 537).

Age

In the original publication² and in later comments^{5,6} it was suggested that the sediments now placed in the Chemoigut Formation were probably younger than 1.2 Myr, but it was stressed that this estimate depended for its accuracy on the geological interpretation. Subsequent work has shown that the relationship of the Chepchuk trachyte (from which the 1.2 Myr date was obtained) to the fossiliferous sediments is not yet established.

The fauna from the Chemoigut Formation (Table 1) is not inconsistent with the original assessment. The *Deinotherium bozasi* Arambourg from the formation would, however, be the youngest known if the K-Ar age of less than 1.2 Myr were accepted. This may prove to be the case, but until additional isotopic dates are available the faunal evidence suggests an age for the formation in excess of 1.34 Myr, which is the date of the latest *D. bozasi* recorded elsewhere (below tuff J, Shungura Formation, Omo⁷).

Palaeomagnetic measurements on material from the baked zone of the Chemoigut sediments lying immediately under the Chesowanja basalt member indicate a reversed polarity (A. Brock, personal communication) and preliminary readings suggest that other levels in the Chemoigut Formation are also reversed (P. Dagley, personal communication). Taking other evidence into consideration, this would place the age of the basalt and the underlying sediments in the Matuyama Reversed Epoch between about 0.7 and 2.4 Myr BP.

The Chesowanja Formation is overlain by the Karau Formation, and a ⁴⁰K-⁴⁰Ar age determination on the Karau trachyte has given a date of 0.54 Myr BP. Micaceous within the tuffaceous member of the Karau Formation in its lacustrine facies, are also potentially dateable. Preliminary palaeomagnetic measurements of the tuffaceous member have produced a number of normal readings (P. Dagley, personal communication).

Palaeoenvironment

The artifact assemblages of Group A have not yielded examples of hand axes or cleavers. They lie under and predate the typical

Table 1 Fauna from the Chemoigut Formation

Mollusca	
Gastropoda	<i>Bellamya</i> sp.
Bivalvia	
Pisces	
Siluriformes	
Reptilia	
Chelonia	
Trionychidae	
Pelomedusidae	
Crocodylia	<i>Crocodylus niloticus</i> Laurenti
Mammalia	
Primates	
Cercopithecidae	<i>Simopithecus</i> sp. sp. indet <i>Australopithecus</i> sp. (2 individuals)
Hominidae	
Proboscidea	
Elephantidae	<i>Elephas</i> c.f. <i>recki</i> Dietrich
Deinotheriidae	<i>Deinotherium bozasi</i> Arambourg
Perissodactyla	
Equidae	<i>Equus</i> sp.
Rhinocerotidae	<i>Ceratotherium</i> sp.
Artiodactyla	
Suidae	<i>Mesochorus</i> sp. <i>Metridiochorus andrewsi</i> <i>Metridiochorus jacksoni</i> probably 2 other species
Hippopotamidae	<i>Hippopotamus</i> c.f. <i>amphibius</i> Linn <i>Hippopotamus</i> sp.
Giraffidae	
Bovidae	
Tragelaphini	<i>Tragelaphus</i> sp.
? Tragelaphini	
Bovini	
Reduncini	<i>Kobus</i> sp.
Alcelaphini	? <i>Megalotragus kattwinkeli</i> Schwarz ? <i>Connochaetes</i> sp. Small species Smaller species
Antilopini	? <i>Antidorcas</i> sp.

Acheulian elements of group B and thus invite comparison with the 'non-biface' Oldowan Industry at Olduvai Gorge. The sediments of the Chemoigut Formation indicate an environment on the margins of a saline lake with a fluctuating water level. Several fresh cores and flakes have a calcium carbonate encrustation of algal origin. It is interesting that the Bed I and lower Bed II Oldowan assemblages of Olduvai also occur around the margins of a saline lake⁸.

The Acheulian of group B is from a subaerial environment on the upper surface of a complex weathered profile. The artifact sites lay to the east of an anticlinal ridge at the foot of a pronounced basalt feature caused by the sharp flexure forming the eastern limb. A lake may have existed only a short distance further to the east as the Acheulian surface is overlain by well bedded, tuffaceous strata, indicating deposition in standing water. A sudden local rise in the water level would have occurred with the influx into the catchment area of large amounts of pumiceous ash from the trachyte centre of Karau which lies 4 km to the South-west.

The piles and spreads of artifacts in group C represent stone working sites and occur upon root cast surfaces in flood plain silts. Adjacent fresh flakes can be fitted together on to the large cores from which they were struck. The industry was based on raw material of phonolite cobbles from anastomosing water-courses of the former Mukutan river system. The artifacts were stratified beneath flood plain silts and wash deposits as the local base level rose. Local breaks in sedimentation occur. One of the most pronounced of these is marked by ferruginous pisoliths. Microlithic tools and flakes in obsidian and other lithologies, together with pottery, were stratified during this phase of flood plain deposition which culminated some 2 m above the horizon of group C. Later incision initiated a cycle of erosion which is still continuing and has produced remnant mesas (Fig. 2). This cycle has re-excavated broad channels to below the level of group C and has produced an intermixing,

on the present day surface, of elements of groups C and D, together with occasional implements in iron. The exact relationship of these artifacts will only be established by careful excavation of *in situ* material.

The main flow of the Mukutan river was diverted towards the north by the Njemps tribesmen 100 or more years ago to irrigate the wash plain for agriculture.

Raw materials

An ongoing study is concerned with identification of the various rock types used by makers of the tools in groups A–D. The earliest tool kits reveal great petrological variety including different trachytes, phonolites and welded tuffs, together with occasional basalts. The Acheulian raw materials of group B are restricted to trachyte with some phonolite and a few small pieces in welded tuff. Group C is almost entirely based on water rounded blocks and cobbles of phonolite.

Various volcanic rocks occur in the area surrounding the artifact sites. Thus it is possible to carry out petrological and geochemical investigations, including trace element analyses, to identify the source rocks of the artifacts. This will permit an estimate of the minimum ranges over which the early stone technologists travelled to collect their raw materials.

The fact that two more individuals of robust australopithecines have been found from horizons yielding abundant artifacts (others are known from Olduvai Gorge and East

Rudolf), poses the question: "Who made the tools?"

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Significance of autogenously regulated and constitutive synthesis of regulatory proteins in repressible biosynthetic systems

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The functional implications of the different modes of regulation have been examined systematically. The results lead to certain predictions. The regulatory protein in repressor-controlled systems is constitutively synthesised. In activator-controlled systems synthesis of the regulatory protein is autogenously regulated. There is favourable agreement between these predictions and published experimental evidence.

REGULATION of specific enzyme synthesis has been most thoroughly characterised in the inducible catabolic systems of enteric bacteria^{1,2}, and in the temperate bacteriophage λ (ref. 3). Regulation of enzyme synthesis in the biosynthetic systems of bacteria has remained more obscure. Among studies on systems of the latter type, those on the tryptophan biosynthetic system in *Escherichia coli* are probably most advanced. Regulation of this operon clearly involves the classical mechanism proposed by Jacob and Monod⁴. Tryptophan, the end product of the pathway, is a corepressor that can combine with an aporepressor molecule to form a "repressor" that binds to the tryptophan operator and blocks transcription^{5–10}. Arginine regulation in *E. coli* may also involve a classical aporepressor^{11,12}, although the evidence is less extensive.

In contrast to the tryptophan and arginine systems, there is no evidence of a classical aporepressor for the histidine and isoleucine–valine biosynthetic systems, although these systems also have been investigated extensively. Consequently, basic assumptions about repressor function have been re-examined critically and other mechanisms have been proposed.

Histidyl-tRNA, rather than histidine itself, has been clearly implicated in repression control of the histidine operon in *Salmonella typhimurium* (see review by Brenner and Ames¹³). Ames and his coworkers^{13–15} suggested that a complex of histidyl-tRNA synthetase (aporepressor) and aminoacylated tRNA^{His} (corepressor) may be a repressor of the histidine operon. In contrast to this suggestion that the synthetase is a negative element in control, Wyche *et al.*¹⁶ reported evidence that the synthetase is a positive element. There is evidence for yet another type of model in which the first enzyme in the histidine biosynthetic pathway (N-1-[5'-phosphoribosyl] adenosine triphosphate:pyrophosphate phosphoribosyltransferase, EC 2.4.2.17) has a role in repression control. Goldberger and coworkers^{17–19} proposed that the first enzyme acts as a negative element in the control. But involvement of the first enzyme as a positive element in the control of the histidine operon also has been suggested^{19,20}.

These and other possible models now can be tested with purified (or partially purified) components in an *in vitro* transcription system and in a coupled transcription–translation system. Blasi *et al.*²¹ reported that the first enzyme blocks *in vitro* transcription of the histidine operon but, surprisingly, histidyl-tRNA was not required for this result. In contrast, Artz and Broach²² obtained evidence that a positive element is required for expression of the histidine operon in the coupled transcription–translation system. Their studies also suggest that transcription of the histidine operon requires translation of its specific mRNA. One of several explanations consistent with these observations is that translation produces a positive factor required for transcription²². Thus, the results so far from these *in vitro*

systems are consistent with the notion that a product of the histidine operon is involved in regulating its own synthesis. In one case, however, this product is thought to be the first enzyme and a negative element in control, whereas in the other, this product is an unspecified positive factor. A combination of the two is also a possibility.

Similar developments have occurred in the study of the isoleucine-valine (*ilv*) biosynthetic system. This system involves multivalent repression^{23,24} in a branched set of pathways²⁵ and is, therefore, more complex than the histidine system. As is the case for the histidine operon, no classical aporepressor has been identified for the isoleucine-valine system. On the basis of biochemical data Hatfield and Burns²⁶ suggested a model of repression control for the *ilvADE* operon in *S. typhimurium* that involves the first enzyme of the isoleucine pathway (threonine deaminase, EC 4.2.1.16), leucyl-tRNA, valine, threonine and isoleucine. According to this model, which now has been modified and extended to cover regulation of the other *ilv* operons in *E. coli* and *S. typhimurium*²⁷, the first enzyme is an aporepressor and the multivalent corepressors are isoleucyl-tRNA, leucyl-tRNA and valyl-tRNA. On the other hand, Levinthal *et al.*²⁸ have described a regulatory mutant of *E. coli* in which the expression of the *ilvADE* operon is diminished and unresponsive to variations in the branched chain amino acids. This phenotype results from a single point mutation in the structural gene for the first enzyme of the isoleucine pathway. Based on these and other genetic results, Levinthal *et al.*²⁸ suggested that the first enzyme is a positive element controlling expression of the isoleucine-valine system.

A defective transducing phage harbouring the *ilv* genes²⁹ is being used for *in vitro* transcription and coupled transcription-translation assays, to study the molecular events in the regulation of the isoleucine-valine biosynthetic system of *E. coli*.

As this brief introduction indicates, there are repressible biosynthetic operons whose regulation involves a classical, constitutively synthesised aporepressor. There also are examples of such systems that involve autogenous regulation, whereby a regulatory protein directly modulates expression of its own structural gene³⁰. Although in no case has it been demonstrated that either of these is the sole mechanism of control, the question arises: what are the functional implications of these two established modes of regulation? This question cannot be answered by comparing directly two representative systems (for example, tryptophan and histidine) because there may be still other elements involved in their control and because the systems are different in many ways that are irrelevant to a comparison of the two modes of regulation *per se*. An answer requires a more controlled comparison in which the two systems are identical in every respect except for the difference in a regulatory mechanism. This is difficult to do experimentally. At present a more practical approach is to compare mathematically models that accurately represent the different types of regulation. Such comparisons have been made and the details, unnecessary here, are available³¹. Before presenting the results it will be useful to enumerate the criteria for functional effectiveness that have been used in comparing the various models.

Criteria for functional effectiveness

Just as numerous chemical and physical criteria make possible characterisation of the molecular components of biological systems, well defined criteria make possible characterisation of the functional behaviour of an integrated system. For simplicity, in the analysis that follows I deal specifically with the regulation of unbranched pathways for the biosynthesis of amino acids. The following criteria for functional effectiveness can be formulated for these systems. It should be noted that none of these criteria

assumes anything about the type of regulatory mechanism involved.

(1) Minimisation of the change in the level of end product as it shifts from one steady state to another in response to a change in demand for end product. This implies that the end product will not be depleted to such an extent that its rate of utilisation is limited when the demand is increased.

(2) Responsiveness to change in the availability of initial substrate. This is essential for the redistribution of common intermediary metabolites that occurs in response to a changing environment when the available carbon is limited.

(3) Reduction of enzyme synthesis when the end product is supplied exogenously. Since the biosynthetic enzymes become superfluous when the end product is available preformed in the environment, the importance of this criterion for cellular economy is obvious.

(4) Stability. Presumably one of the prime functions of pathways synthesising amino acids is to provide a relatively constant supply of their end products for protein synthesis. An effective system would not oscillate wildly, starving the organism for end product during one phase and over-producing and wasting it in the next. The impaired growth of mutants exhibiting such instability is readily apparent³².

(5) Temporal responsiveness to change. The regulatory mechanism should enable the system to respond quickly to changes in its environment.

(6) Insensitivity to perturbations in the structure of the system itself. Small changes in the system may be the result of mutations in structural or regulatory genes, errors in transcription or translation of the genetic information, or physical influences such as temperature shifts. These changes tend to exert a deleterious effect on the cell and, as Sonneborn suggested³³, cells "buffered" against such harmful effects are likely to have a selective advantage.

Repressor-controlled systems

Models of classical and autogenous regulation are represented schematically in Fig. 1. In the classical Jacob-Monod model the structural gene for the regulatory protein is located in a separate transcriptional unit and is itself unregulated, whereas in the model of autogenous regulation one of the protein products of the operon is the regulator, which therefore directly controls expression of its own structural gene. For purposes of comparison these models are assumed to be equivalent except for their differences in regulatory interaction.

These two models can be compared for a wide variety of conditions, and because there are several criteria to consider, a meaningful tabulation of all the results presents a problem. Figure 2, however, is a rather simple diagram that conveniently summarises many of these results. In this plot the vertical axis represents the strength of the contribution of end product to the regulation while the horizontal axis represents the strength of the autogenous contribution. Each point in this two-dimensional space represents a different system with distinctive properties. Nevertheless, this space can be divided into subclasses of systems that have similar properties.

In repressible systems that utilise a repressor mechanism, the end product has a negative effect on transcription, and the autogenous contribution is also negative. Since both contributions are negative, only the lower left hand quadrant of Fig. 2 needs to be considered. The vertical axis in this quadrant is the locus of points representing systems with constitutively synthesised repressor, since the autogenous contribution is zero. Line *b* represents another important classification. The position of this line is determined by analysing the local stability of these models. All systems represented by points above line *b* are stable (if perturbed momentarily, they will return to their pre-disturbance condition), whereas all systems represented

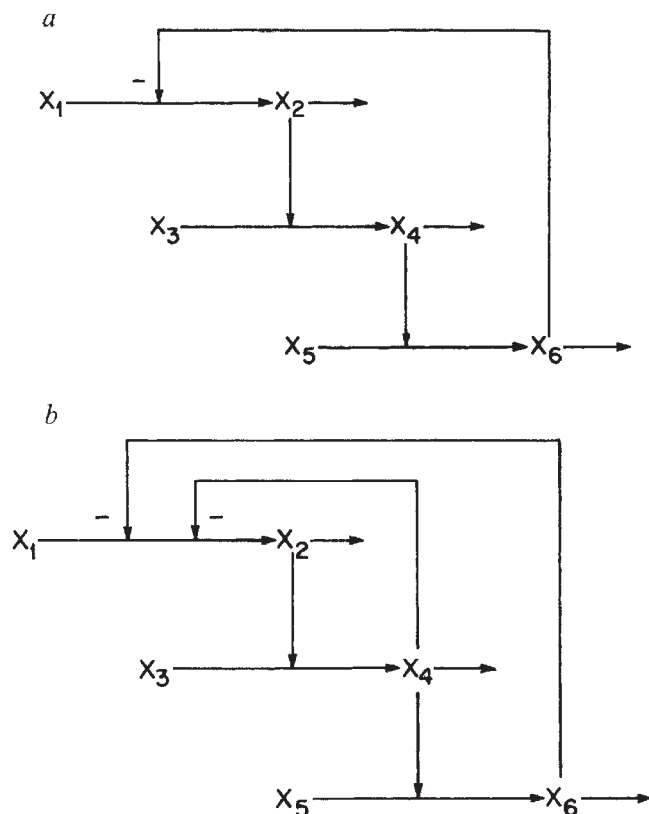


Fig. 1 Schematic models of repressible biosynthetic systems. *a*, Model with constitutive synthesis of the regulatory protein, which is not explicitly indicated. *b*, Model with autogenously regulated synthesis of the regulatory protein. X_1 , Weighted sum of the nucleotide pools; X_2 , specific messenger RNA; X_3 , weighted sum of the amino acid pools; X_4 , enzymes of the biosynthetic pathway (plus regulator in the case of autogenous regulation); X_5 , substrate, and X_6 , product/modifier. The horizontal arrows indicate chemical transformations at the mRNA, enzyme and metabolite levels. The vertical arrows represent modifier or catalytic influences (the sense of the influence is positive unless otherwise indicated). Except for the differences in regulation these models are assumed to be identical.

below this line are unstable (they will not return to their predisturbance condition).

Lines, such as *c*, *d* and *e*, that radiate from a single point on this plot are lines of equivalence with respect to the first three criteria for functional effectiveness. All systems whose regulatory parameters have values that determine points on a given line of equivalence have: (1) the same steady-state response to a change in demand for the end product, (2) the same steady-state response to a change in substrate availability and (3) the same decrease in enzyme synthesis for a given increase in the concentration of extracellular end product. The slope of the line determines the magnitude of the above three responses. Systems represented on a steeper line of equivalence, function more effectively according to the first and third criteria but less effectively according to the second.

Systems for which the second criterion is of primary importance are represented by lines of equivalence with low slope, for example, line *c*. As previously noted, all systems on such a line have identical responses according to the first three criteria. Comparisons on the basis of stability, the fourth criterion, can be made by noting the relative distances of points on line *c* from the boundary of instability, line *b*, in Fig. 2. Since lines *c* and *b* diverge to the left, systems with greater strengths of autogenous regulation are represented further from the boundary of instability than is the equivalent system with constitutive

synthesis of repressor. Consequently, this latter system is the least stable of all the systems represented on the line of equivalence *c*.

Systems for which the first three criteria are of equal importance are represented by lines of equivalence with intermediate slope, such as line *d* in Fig. 2. All the systems represented on this line have identical responses according to the first three criteria. They also have the same degree of stability since line *d* is parallel to the boundary of instability, line *b*.

Similarly, systems for which the first and third criteria are of primary importance are represented by lines of equivalence with steep slope, for example, line *e* in Fig. 2. These systems are equivalent as judged by the first three criteria, but the systems with autogenously regulated synthesis of repressor must be considered inferior to the equivalent one with constitutive synthesis of repressor according to the fourth criterion because autogenous regulation now is clearly a destabilising influence.

The two types of repressor-controlled systems are examined in Fig. 3 on the basis of temporal responsiveness, the fifth criterion for functional effectiveness. The three panels in this figure correspond to the three different classes of equivalence represented by lines *c*, *d* and *e* in Fig. 2. In the top panel (*c*) the response labelled 0.0 corresponds to the system with constitutive synthesis of repressor. The system is in a steady state with concentrations normalised to unity before time zero. At $t=0$ the concentration of extracellular end product is increased and then maintained at an elevated level throughout the experiment. The concentration of intracellular end product is plotted as a function of time. Similar responses are plotted for a series

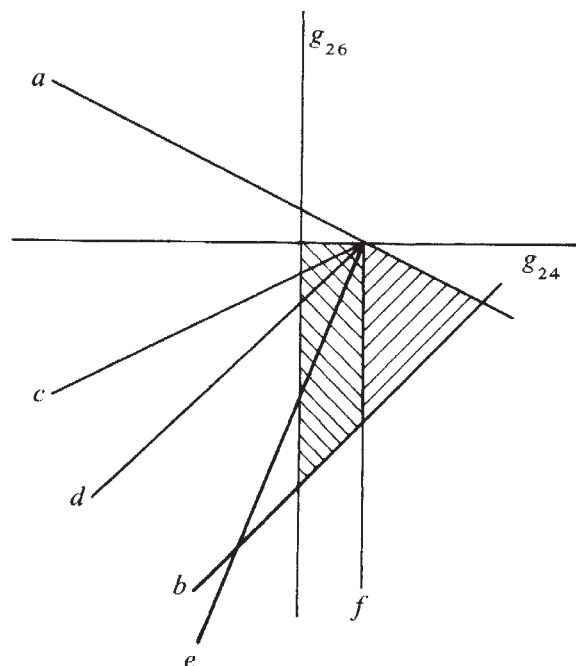


Fig. 2 Graphical comparison of systems with alternative types of regulation. The vertical axis represents the strength of the product's contribution to the regulation of transcription and the horizontal axis represents the strength of the autogenous contribution to this regulation. *a* and *b*, Boundaries of instability. *c*–*f*, Lines of equivalence with respect to the first three criteria for functional effectiveness. The vertical axis is the locus of points representing systems with constitutive synthesis of either activator or repressor. The shaded areas to the right of this axis are the locations of points representing stable systems with autogenously regulated synthesis of activator. Similarly, the area above line *b* in the lower left hand quadrant represents stable systems with autogenously regulated synthesis of repressor. (See text for further discussion.)

of systems with different strengths of the autogenous contribution to the regulation. Clearly, autogenous regulation decreases the time required for the system to settle into the new steady state. The same is true in Fig. 3*d*. In Fig. 3*e*, however, autogenous regulation actually increases the response time.

A comparison on the basis of the sixth and final criterion for functional effectiveness shows that the sensitivities to external perturbations of the systems with autogenously regulated synthesis of repressor are less than or equal to those of the system with constitutive synthesis of repressor.

On the basis of the comparisons discussed above one can draw the following conclusions. If the second criterion for functional effectiveness is of primary importance, then systems with autogenously regulated synthesis of repressor will have a selective advantage. The behaviour of these systems is superior to that of systems with constitutive synthesis of repressor on the basis of the second, fourth, fifth and sixth criteria, but inferior with regard to the first and third. But if the first and third criteria for functional effectiveness are of primary importance, as seems more reasonable for biosynthetic systems, then systems with constitutive synthesis of repressor will have the selective advantage. The behaviour of these systems is superior to that of systems with autogenously regulated synthesis of repressor on the basis of criteria one, three, four and five, but inferior with regard to two and six. These conclusions will be discussed in relation to available experimental evidence in a later section.

Activator-controlled systems

The models represented in Fig. 1 also can be used for the comparisons in this section. Systems with constitutive synthesis of an activator protein can be represented directly by the model in Fig. 1*a*, whereas systems with autogenously regulated synthesis of activator can be represented by the model in Fig. 1*b* provided the negative sign previously associated with the repressor is changed to a positive one, implying activation. Since the autogenous contribution to the regulation now is positive, only the lower right hand quadrant of Fig. 2 need to be examined. The systems with constitutive synthesis of activator are represented along the vertical axis. As in the previous section, line *b* represents the lower boundary of stability. In addition, there is an upper boundary of stability given by line *a*. As in the previous section, lines, such as *c*, *d*, *e* and *f*, that pass through a common point on this plot are lines of equivalence with respect to the first three criteria for functional effectiveness.

Systems for which the second criterion is of primary importance again are represented by points on line *c*. These systems are equivalent with respect to the first three criteria, but since line *c* diverges to the left from the boundary of instability, line *b*, the system with constitutive synthesis of activator is more stable than the equivalent systems with autogenously regulated synthesis of activator. Systems for which the first three criteria are of equal importance are represented by points on a line of equivalence with intermediate slope, such as line *d*. All the systems represented on this line have identical responses according to the first three criteria, and according to the fourth criterion as well, since lines *b* and *d* are parallel. Similarly, systems for which the first and third criteria are of primary importance are represented by a steeper line of equivalence, such as *e* in Fig. 2. These systems are also equivalent by the first three criteria, but those with autogenously regulated synthesis of activator must be considered superior to the equivalent one with constitutive synthesis of activator according to the fourth criterion because autogenous regulation is a stabilising influence. Thus, the effect of autogenous regulation on the stability of activator-controlled systems is

just the opposite of that found to be true for repressor-controlled systems.

The results in Fig. 4 show that autogenous regulation also has opposite effects on the response times of repressor-controlled and activator-controlled systems. The three panels in this figure correspond to the three different classes of equivalence represented by lines *c*, *d* and *e* in Fig. 2. The response time is increased by autogenous regulation in Fig. 4*c* and *d*. In Fig. 4*e*, however, the response time is decreased by autogenous regulation.

A comparison on the basis of system sensitivity, the sixth criterion, shows that the sensitivities to external perturbations of the system with constitutive synthesis of activator always are less than or equal to those of the equivalent systems with autogenously regulated synthesis of activator.

The following conclusions can be drawn from the comparisons in this section. If the second criterion for functional effectiveness is of primary importance, then systems with constitutive synthesis of activator will have a selective advantage. These systems are superior to systems with autogenously regulated synthesis of activator by the second, fourth, fifth and sixth criteria, but inferior according to the first and third. If, however, the first and third criteria for functional effectiveness are of primary importance, as seems more reasonable for biosynthetic systems, then systems with autogenously regulated synthesis of activator will have the selective advantage. These systems are

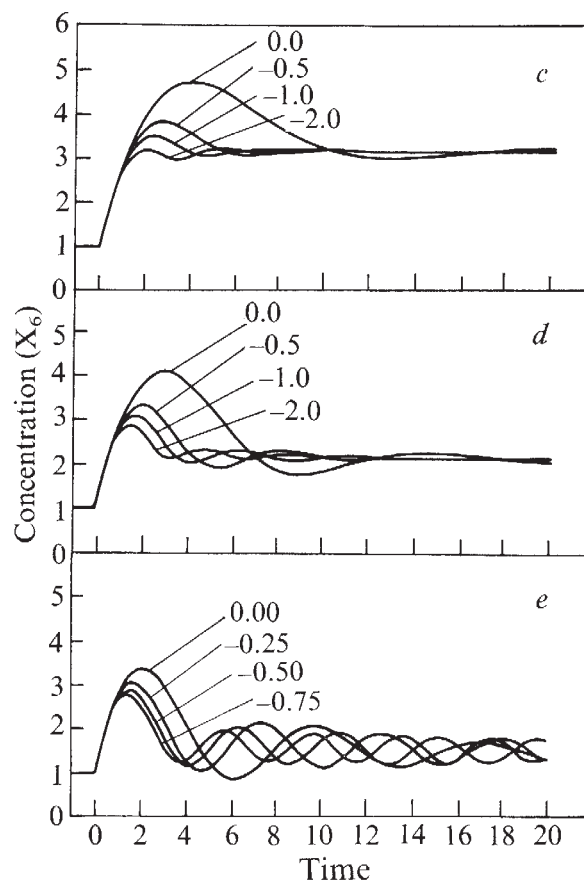


Fig. 3 Temporal responsiveness of systems with autogenously regulated synthesis of repressor. *c*, *d* and *e* correspond to systems in the three different equivalence classes represented by lines *c*, *d* and *e* in Fig. 2. The numbers associated with the individual curves are values of g_{24} , representing the strength of the autogenous contribution to regulation. The corresponding values for the parameter g_{26} are chosen so that the points representing the different systems within a class all lie on the same line of equivalence. Curves with $g_{24} = 0$ represent systems with constitutive synthesis of repressor. (See text for further discussion.)

superior to systems with constitutive synthesis of activator on the basis of criteria one, three, four and five but inferior with regard to two and six.

(Re-examination of the lower right-hand quadrant in Fig. 2 shows that only a portion of the allowable systems have been considered in the preceding comparisons. The remaining systems with autogenously regulated synthesis of activator exhibit two classes of behaviour for which there is no counterpart among the other types of regulatory systems considered here. Line *f* represents the class of systems for which there is zero depletion of end product in steady state after an increase in demand for end product. The class of systems represented by points within the shaded triangular region to the right in Fig. 2 have the unique property of responding to an increased demand for end product by increasing the level of that end product—a response to demand that is the inverse of the usual one. Whether such behaviour has biological significance is unclear and it will not be discussed further here. A more detailed analysis is given in ref. 31.)

Predictions and observations

As previous sections of this article show, the effectiveness of activator-controlled and repressor-controlled operons is changed in opposite ways if the regulator protein is, in addition, autogenously regulated. In systems for which the

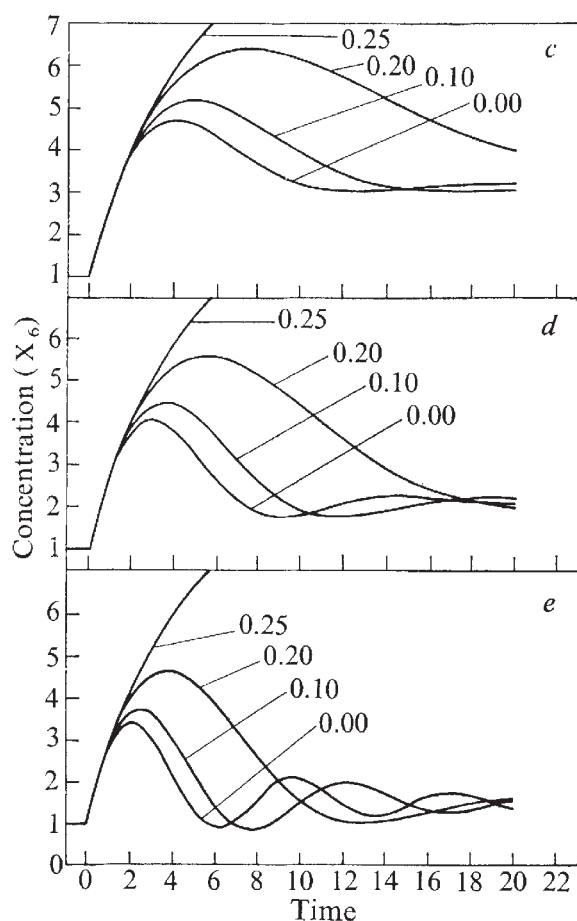


Fig. 4 Temporal responsiveness of systems with autogenously regulated synthesis of activator. *c*, *d* and *e* correspond to systems in the three different equivalence classes represented by lines *c*, *d* and *e* in Fig. 2. The numbers associated with the individual curves are values of g_{24} , representing the strength of the autogenous contribution to regulation. The corresponding values for the parameter g_{26} are chosen so that the points representing the different systems within a class all lie on the same line of equivalence. Curves with $g_{24} = 0$ represent systems with constitutive synthesis of activator. (See text for further discussion.)

first and third criteria for functional effectiveness are most important, as is likely for repressible biosynthetic systems, autogenous regulation of the regulator protein improves the performance of activator-controlled systems and degrades that of repressor-controlled systems. Thus, biosynthetic systems governed by a repressor are not expected to use autogenous regulation, while those governed by an activator can be predicted to involve such regulation. These predictions were deduced from differences inherent in the classical and autogenous modes of regulation, other things being equal. Present information concerning actual, specific operons is insufficient to yield such generalisations through induction from individual cases. Nevertheless, the experimental evidence is consistent with the above predictions in the few cases where data that can be analysed are available.

In the case of the tryptophan operon in *E. coli*, the best documented example of a biosynthetic system involving control by a repressor, there is no evidence for autogenous regulation of repressor. The structural gene for the tryptophan repressor is located outside the known transcriptional units under its control³⁴, so the simplest form of autogenous regulation is excluded. Nevertheless, a more complex, regulon model, in which the repressor modulates expression of its own structural gene in parallel with that of the tryptophan operon, cannot be excluded rigorously since repressor levels have not been compared in strains with the tryptophan operon repressed and derepressed.

In the case of the arginine system in *E. coli*, which also seems to involve control by a repressor, there is no evidence for autogenous regulation of repressor. Although the arginine system itself constitutes a regulon with several unlinked transcriptional units regulated in parallel³⁵, the structural gene for the repressor is not linked to any of these³⁴ and there is no evidence that its expression is subject to modulation by the level of arginine within the cell.

On the other hand, the clearest example of a biosynthetic system involving control by an activator is the isoleucine-valine system in *Saccharomyces cerevisiae* and in this case the activator is autogenously regulated^{36,37}. With an extensive collection of mutants in the structural gene for the first enzyme of the isoleucine pathway (threonine deaminase), Bollon³⁷ has constructed a fine-structure map and demonstrated that this protein is multifunctional; mutations affecting its activator function overlap with mutations affecting its catalytic function.

For all three operons discussed above the evidence is consistent with the predictions based on the analysis of autogenous regulation. For other biosynthetic systems the experimental evidence is more ambiguous. For example, in *E. coli* there is strong experimental evidence that the first enzyme of the isoleucine pathway is involved in the autogenous regulation of the isoleucine-valine system, but whether it functions as repressor or activator is unclear. The prediction based on the analysis in the preceding sections is that some product of the operon is an activator, in agreement with the data of Levinthal *et al.*²⁸. Similarly, in *S. typhimurium* there is evidence consistent with the idea that some product of the histidine operon is involved in autogenous regulation of the system, but whether this product is an activator or a repressor is controversial. Again, the prediction is that this product is an activator, in agreement with the data of Artz and Broach³². (The conflicting evidence for the nature of the regulators of these operons, which was referred to in the introduction, might be reconciled more easily if they were in fact bi-regulators rather than activators. It is easier to conceive of a biregulator changing physical state, as a result of mutation or environmental conditions, and exhibiting the properties of either repressor or activator than to imagine an activator doing this. Nothing in the analysis and predictions given above would change substantially if activators

were considered biregulators analogous to the arabinose C-gene product, which is primarily an activator in function but also has repressor attributes. The arguments are given in ref. 31.)

The tryptophan and arginine biosynthetic systems have been described in terms of a classical, Jacob-Monod model with constitutive synthesis of regulator. On the other hand, the histidine and isoleucine-valine biosynthetic systems have been described in terms of an autogenously regulated model with an enzyme/regulator directly involved in modulating expression of its own structural gene. These two models, however, need not be mutually exclusive. A combination of the two modes of regulation might exist, possibly with one or the other mode playing a dominant role in some systems. The results in the preceding sections suggest that the most promising of such combinations would be constitutive synthesis of a repressor and autogenously regulated synthesis of an activator. Is there any experimental evidence for such a combination?

There is evidence for multiple control mechanisms in most of the biosynthetic systems that have been well studied. Mutants with a chain-termination codon early in the structural gene for the first enzyme or with this gene deleted have been described in the histidine¹⁹ and isoleucine-valine³⁸ biosynthetic systems of enteric bacteria. In each case, the system continues to be regulated in an apparently normal manner, at least by the usual all-or-none tests for regulatory function. Thus, mutations that eliminate the autogenous mode of regulation permit another, possibly the classical mode, to become manifest.

A better example is provided by the tryptophan operon of *E. coli*. In this system, where there is clear evidence for the classical mode of regulation involving a constitutively synthesised repressor, there also is evidence suggesting that the first enzyme of the tryptophan pathway (anthranilate synthetase, EC 4.1.3.27) is involved in regulating expression of the operon³⁹. Somerville and Stetson^{40,41} reported additional evidence for involvement of the first enzyme in regulation of the tryptophan operon and suggested that this enzyme is normally an activator or positive element in the control, which agrees with the above prediction. When the structural gene for the first enzyme is deleted, the tryptophan operon continues to be regulated in an apparently normal manner, again by the usual all-or-none tests for regulatory function⁴².

In addition to the two modes of regulation discussed above there are indications of novel control mechanisms⁴³⁻⁴⁵, at least some of which involve sites on the DNA between the promoter-operator region and the first structural gene of the operon^{44,45}.

In systems for which the first and third criteria for functional effectiveness are relatively unimportant, the results are just the reverse of those discussed above. Autogenous regulation of the regulator protein improves the performance of repressor-controlled systems and degrades that of activator-controlled systems. Although it seems unlikely that any repressible biosynthetic system would fall into this particular class, there are systems of other types that might be considered non-biosynthetic, that do fit this category. For example, Sompayrac and Maaløe⁴⁶ have proposed a model for regulating the concentration of the protein(s) believed to be involved in the initiation of DNA replication. The regulator protein in this model is autogenously regulated and functionally autonomous, that is, it does not require the participation of an effector molecule in its regulatory function. Although not a repressible biosynthetic system in the conventional sense of the term, formally this model is a special case of that represented in Fig. 1b with $g_{26} = 0$. Accordingly, such systems would be represented along the horizontal axis in Fig. 2. Sompayrac and Maaløe⁴⁶ pointed out several advantages for their model involving a repressor whose synthesis is autogenously

regulated. The results in the preceding sections suggest that the behaviour of such systems is optimal with respect to stability, temporal responsiveness and insensitivity to perturbations in the structure of the system itself. Dennis and Nomura⁴⁷ have indicated that in *E. coli* a similar mechanism of autogenous regulation might be involved in control of ribosomal protein genes.

Perhaps it should be re-emphasised here that the predictions made throughout this article are based on a particular weighting of the different criteria for functional effectiveness. If this choice were incorrect, then the predictions could be wrong, but clearly for reasons that have nothing to do with the general validity of the approach used.

In conclusion, the expression of biosynthetic operons in enteric bacteria is undoubtedly controlled by several different mechanisms acting in concert. This has made it difficult to study regulation *in vivo* where all the elements may interact or contribute in various degrees to produce the end result. The identity of the elements participating in the regulation and the nature of their roles at the molecular level can only be established rigorously *in vitro*. An understanding of the intact regulatory system, however, will require knowledge of the functional implications at the physiological level for each molecular mechanism and, eventually, integration of the individual mechanisms into an accurate model of the intact system. With progress being made in each of these areas, the understanding of repressible biosynthetic operons should soon be as thorough as it is of the simpler inducible catabolic operons.

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letters to nature

Soft X-ray search of centre of Cygnus Loop

THE Mullard Space Science Laboratory equipment on the Copernicus satellite has been used to search, with negative results, for evidence of a compact object in the centre of the Cygnus Loop supernova remnant.

Rocket measurements¹ indicate that a central object exists, and contributed, at the time of observation, about 4% of the X-ray flux from the entire loop. This object may be variable^{1,2} in intensity, and may even be pulsing¹. Reanalysis of earlier rocket data³ gives statistically more significant evidence for pulsation, but at a different frequency, which would be consistent with a compact object which is not a pulsar. The same data require, however, that the pulsing object be roughly equal in brightness to the integrated brightness of the rest of the nebula at the relevant energy, 0.28 keV, at the time of observation, while other rocket data^{4,5} show that this is certainly not the case at particular other times. The availability of a Channel Electron Multiplier (CEM) detector, sensitive at the relevant energy (0.28 keV), on board the Copernicus satellite, has provided the opportunity to observe the centre portion of the Cygnus Loop and to test whether a central object does, from time to time, radiate as strongly at 0.28 keV as rocket data³ suggest.

The CEM has been described by Margon *et al.* (see ref. 6 and references therein). The field-of-view of the CEM is 15' full width at half maximum. Since the exact position of the central object is not known, a set of nine observing sections, centred on the rocket¹ position, were chosen so that there was

a slight degree of overlap. These nine positions are given in Table 1.

The observations were made on May 19, 1974. Three spacecraft orbits were required to observe the nine sections. Sections, 1, 2, 3 were covered from 1349 to 1422 UT; 6, 5, 4 from 1530 to 1600 UT; and 7, 8, 9 from 1709 to 1739 UT. The observations could only be made while the satellite was in the shadow of the Earth since the CEM is also sensitive to Lyman alpha (L α) radiation. During the periods when the satellite was in sunlight, the CEM was turned off. (Margon *et al.*⁶ note that in spite of the L α background, the telescope is known to respond normally to low energy X rays; in particular Sco X-1 has been observed.) Each data acquisition interval lasted 62.5 s. From six to ten observations were made in each section.

Figure 1a presents plots of the raw data as received from the satellite. Three points are immediately apparent: (1) each plot displays a characteristic parabolic shape, due to CEM sensitivity to geocoronal L α radiation; (2) a systematic decrease in this background occurs on successive orbits, clearly due to instrumental effects; and (3) no sharp break occurs between any of the three sections on each of the three orbits, as would be expected if a strong source of 0.28 keV X rays were present. If the central source were present, at the greatest intensity observed³, we would expect that one of the sections would display a 'discontinuity' of about 735 counts over the L α background, given the known sensitivity⁶ to X rays of the CEM. A lower flux would result in a smaller discontinuity. The data show that if such a source was present in the field of view, it was not of high intensity at the time of observation.

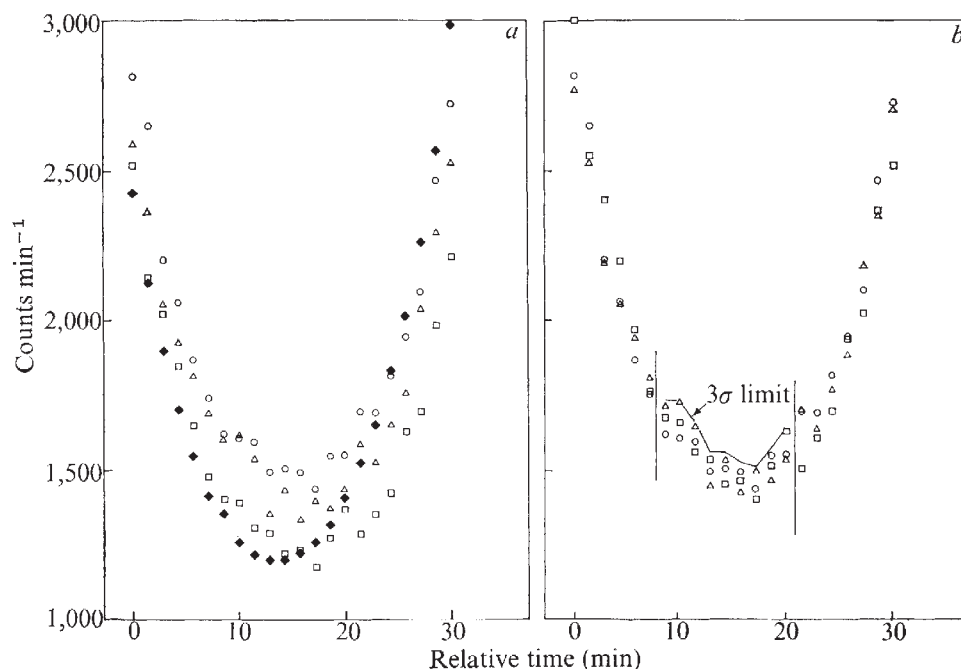


Fig. 1 *a*, Signal received from directions near the centre of the Cygnus Loop nebula on three orbits of the Copernicus satellite. The data are shown in raw form, and give no evidence for any soft X-ray emission. Also shown is the relative expected background of L α radiation, calculated by Dr R. Meier of the US Naval Research Laboratory. *b*, The data of *a* are shown, processed to remove instrumental effects. This permits the determination of a 3 σ upper limit on any possible X-ray source, which is about 80 times smaller than the highest reported observed intensity. $\circ$, First orbit; $\triangle$, second orbit; $\square$, third orbit; $\blacklozenge$, theoretical L α .

The change in sensitivity of the CEM would be unimportant if an obvious discontinuity in the data was apparent. One could say in those circumstances that a source was present, and one could determine in which section it appeared. In obtaining an upper limit for the source, however, the change in sensitivity becomes critical. Since each orbit displays the same characteristic pattern, we feel safe in believing that each curve represents only La background. We have therefore transformed orbit two and orbit three to orbit one by using a simple *ad hoc* function that corrects for the CEM drift. Almost any transformation will be acceptable if it results in a variance at each point comparable to some typical variance of the data points on one orbit about some smooth function representing the La background.

Figure 1b represents the result of using a correction consisting simply of multiplication of the data by an orbit-dependent constant. The constant for each orbit was chosen so that

$$\sigma^2 = \sum_{i=1}^N \sigma_j^2 = \sum_{j=1}^N \sum_{i=1}^N \left\{ \frac{(x_{ij} - \bar{x}_j)^2}{2} \right\} \quad (1)$$

was a minimum where $\bar{x}_j$ is the average of the three transformed points, x_{ij} is the transformed value of point j in orbit i , and N observations are made during one orbit.

Table 1 Observed positions in the Cygnus Loop

Section	RA (h min)	Position (1950) (° ')	Dec. (° ')
1	20 50.5		+30 53
2	20 49.7		30 53
3	20 48.9		30 53
4	20 50.5		30 43
5	20 49.7		30 43
6	20 48.9		30 43
7	20 50.5		30 33
8	20 49.7		30 33
9	20 48.9		30 33

The minimum σ^2 was obtained by multiplying the observations of orbit 2 by 1.07 and of orbit 3 by 1.19, and each σ_j^2 was indeed comparable to the scatter among individual points observed on a single orbit, indicating that no more complicated correction procedure is justified.

A three sigma upper limit on the intensity of any soft X-ray source may be obtained by adding a source of ϵ photons per 62.5 s to the background La radiation in one of the sections in Fig. 1b. The value of ϵ is increased until the quantity

$$\chi^2 = \sum_{j=1}^n \frac{((\bar{x}_j + \epsilon) - \bar{x}_j)^2}{\sigma_j^2} \quad (2)$$

(where n is the number of observations in a section), becomes so large that one can say with 99.74% certainty that the data in that section include something more than La radiation. The line in Fig. 1b represents this three sigma limit.

Knowing the relevant parameters of the CEM, a three sigma upper limit on the intensity of the X-ray source of 5.7 photons $(\text{cm}^2 \text{ s keV})^{-1}$ is obtained. This is a factor 80 below the highest reported rocket intensity³.

This negative result could indicate that the X-ray source was simply not in its high-intensity mode at the time of observation, or could arise because the source is at some other location in the Loop. The possibility also remains, of course, that the evidence for a central object¹, intensity variations^{1,2}, and pulsations with very high intensity³, is misleading and no such object exists. If it does exist, it is clear that it is strongly time-dependent, and it would be valuable to monitor the assumed source position frequently. Unfortunately noise

bursts on the CEM channel have precluded additional observations.

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Proper motions of six pulsars

SEVERAL authors have suggested that pulsars may be high velocity objects. Prentice¹ found, from a study of the wide distribution of the distances of pulsars to the galactic plane, that their initial velocities may have been as high as 100 km s⁻¹, and Michel² obtained a similar figure for runaway objects formed by the collapse of core fragments in supernova events. Tademaru and Harrison³ proposed a mechanism for the acceleration of pulsars, by asymmetric radiation, to velocities of 1,000 km s⁻¹. Only rather meagre direct experimental evidence for high velocities exists, however. This evidence is derived from three different types of observation. First, Lang and Rickett⁴, Galt and Lyne⁵, and Slee *et al.*⁶ have deduced velocities of between ~ 40 km s⁻¹ and ~ 600 km s⁻¹ from measurements of the velocity of the interstellar scintillation pattern, found by comparing the scintillation observed at two widely separated stations. These conclusions rely, however, on the pattern being stable in time. In some cases this is clearly not so, and in particular Uscinski⁷ has shown how shear within the scintillating medium can produce an apparent velocity which bears little relation to that of the pulsar. These measurements must therefore be treated with caution. Second, pulse-timing studies by Manchester, Taylor and Van⁸ gave an apparent motion of $0.58 \pm 0.22''$ for PSR1133+16, corresponding to a velocity of 380 km s⁻¹. Finally, the transverse velocity of the optical object identified with the Crab Nebula pulsar, PSR0531+21, is 100 km s⁻¹ as deduced from the proper motion and estimated distance⁹.

Here we report measurements on six pulsars which seem to have motion velocities at least five times greater than the formal standard errors. Measurements were made on a radio link interferometer of 127-km baseline, using the Mk 1A 76-m radio telescope at Jodrell Bank and a 25-m

Table 1 The proper motions observed for six pulsars

Pulsar	$\alpha \cos \delta$ ($'' \text{ yr}^{-1}$)*	$\dot{\delta}$ ($'' \text{ yr}^{-1}$)*	$\dot{r}$ ($'' \text{ yr}^{-1}$)*	Position angle (degrees)*	Distance (pc)	Velocity (km s^{-1})	Angle ϕ between r and galactic plane ($^\circ$)
0329+54	0.011 ± 0.006	-0.008 ± 0.004	0.014 ± 0.004	126 ± 16	2,500	166	$+1.5 \pm 16$
0823+26	0.050 ± 0.014	-0.125 ± 0.010	0.135 ± 0.010	158 ± 6	649	416	$+5 \pm 6$
0834+06	-0.017 ± 0.018	0.049 ± 0.014	0.052 ± 0.014	-19 ± 20	430	106	$+8 \pm 20$
1133+16	-0.061 ± 0.036	0.360 ± 0.036	0.365 ± 0.036	-10 ± 8	162	281	$+26 \pm 8$
1237+25	-0.098 ± 0.018	0.027 ± 0.014	0.102 ± 0.018	-75 ± 8	308	149	-33 ± 8
2016+28	0.003 ± 0.012	0.020 ± 0.008	0.020 ± 0.008	9 ± 26	473	45	-47 ± 26

* $\pm 2\sigma$

antenna at Defford, Worcestershire, used by arrangement with the Royal Radar Establishment, Malvern. The observing frequency was 408 MHz, with an operating bandwidth of 4 MHz. A phase reference technique similar to that described by Peckham¹⁰ was used, in which the pulsar and a small diameter background radio source are observed simultaneously within the same telescope beam. The relative phases of the two objects are compared at different epochs and, assuming that the background source is extragalactic, any change in the relative phase is then attributed to a proper motion of the pulsar.

Observations were made in November 1973, July 1974, November 1974 and April 1975. Seven pulsars were observed in 1973, and these and a further seven in the subsequent sessions. At each epoch, each pulsar was observed for as wide a range of hour angles as possible when its elevation was greater than 10° . If time permitted, each observation was repeated.

Table 1 shows the proper motions tabulated in both celestial coordinates and polar coordinates. The velocities given in the final column were calculated using the distances presented in the penultimate column. These distances were obtained from the dispersion measures, assuming an average electron density of 0.03 cm^{-3} (refs 11 and 12), except in the case of PSR0329+54, for which absorption measurements place the pulsar on the near side of the Perseus arm of the galaxy (R. S. Booth and A. G. Lyne, unpublished). This use of the dispersion measure is somewhat uncertain, but we believe that it is justified since Prentice and ter Haar¹³ found no regions of high ionisation along the line of sight to any of these pulsars. We have not used their distance estimates directly, because of the rather high mean electron density (0.05 cm^{-3}) used in their model. The discrepancies are not large, however.

The uncertainties quoted in Table 1 are all formal 2σ errors, and these are a function of the intensity of the source and the pulsar, and also of the observing time and hour angle coverage. These errors should be treated with caution, since ionospheric and interstellar scintillation effects can cause fluctuations in the relative-phase curves on time scales comparable with the length of an individual observation. Although the effects of these scintillations will average out as a larger number of observations are made, with the data obtained so far we do not believe the uncertainties can be much less than $0.01'' \text{ yr}^{-1}$. In the case of PSR0329+54, however, the measurements are expected to have less uncertainty, because it is a relatively intense pulsar with a strong background source, it was observed at high elevations, and several repeat observations were made.

The results for the two pulsars PSR0329+54 and PSR1133+16 show some agreement with previous measurements. In the case of PSR0329+54, the measured position angle of $126 \pm 16^\circ$ agrees well with the value of $115 \pm 15^\circ$ found by Galt and Lyne⁵ from scintillation observations. This supports their second interpretation: that it is the pulsar, rather than the diffraction pattern, which is moving with high velocity. They deduce a velocity which is more than a factor of two greater than the present measurement, but

this could be explained if the diffracting screen were situated slightly nearer the pulsar, or if the distance has been underestimated. Our result for PSR1133+16 agrees, within the errors in both magnitude and direction, with that of Manchester, Taylor and Van⁸.

The data in Table 1, together with that for the Crab Pulsar, PSR0531+21, show typical transverse velocity components in the range $100\text{--}200 \text{ km s}^{-1}$. Thus we may deduce that the resultant velocities are high, typically $150\text{--}300 \text{ km s}^{-1}$, although none is as high as the $1,000 \text{ km s}^{-1}$ suggested by several authors¹⁻³. It must be remembered, however, that we are, as yet, dealing with very small numbers of pulsars, and also that there are still some uncertainties in these velocities because of the uncertainties in the pulsar distances.

It is widely believed that pulsars originate in population I type objects which lie within about 100 pc of the galactic plane, and that their high velocities then carry them to heights of several hundred parsecs during their lifetimes. This picture is weakly supported by the data presented in column 8 of Table 1 which shows the angle ($-90^\circ < \phi < 90^\circ$) between the velocity vector $\dot{r}$ and the galactic plane. A positive sign indicates that the apparent motion is away from the galactic plane. It can be seen that three of the pulsars (PSR0823+26, PSR0834+06 and PSR1133+16) are probably moving away from the plane; the direction of motion of PSR0329+54 is not established accurately enough to ascertain whether it is receding from or approaching the plane, and PSR2016+28 and PSR1237+25 seem to be approaching the plane. PSR0329+54 and PSR2016+28 lie within the population I distribution, however, and PSR1237+25 is situated at such a high galactic latitude (86.5°) that motion towards or away from the plane is likely to be dominated by the unknown radial component of velocity. The unknown radial components of velocity of the other pulsars also affect their apparent motions with respect to the galactic plane so that the evidence for recession from the plane is not very strong.

Manchester, Taylor and Van⁸ have estimated a 'kinetic age' for PSR1133+16 by projecting back the apparent motion on the assumption that it started from the galactic plane and that it now has no radial component of velocity. The pulsar velocity should be substantially unaffected by the gravitational attraction of the galactic plane during the pulsar lifetime since the oscillation period of a star across the plane is in excess of 10^8 yr in the region of the Sun. Table 2 shows the 'kinetic ages' for the three pulsars which clearly seem to recede from the galactic plane together with the 'rotational age' derived from observations of changes in

Table 2 Kinetic and rotational ages of three pulsars

Pulsar	Kinetic age ($\text{yr} \times 10^{-6}$)	Rotational age ($\text{yr} \times 10^{-6}$)
0823+26	11	5
0834+06	14	3
1133+16	3.4	5

pulsar periods ($P/2\dot{P}$). The 'kinetic ages' should be regarded as probable upper limits to the true ages of the pulsars since the ages would be significantly reduced if the pulsars had radial components of velocity as small as $+60 \text{ km s}^{-1}$. Because of this, these 'kinetic ages' are not sufficiently accurate to test a recent suggestion by Lyne, Ritchings and Smith¹⁴ that the 'rotational age' may be a substantial overestimate of the true age, particularly in the case of old pulsars.

It is worth noting that the proper motions which have been determined, so far, for pulsars—the six reported here and also the Crab Nebula pulsar, PSR0531+21 (ref. 9)—tend to lie parallel to the galactic plane rather than perpendicular to the plane. The probability of this occurring by chance is quite small, although so few measurements are available that the significance is difficult to assess.

Continuation of this work over longer time scales will yield improvements in accuracy, and provide measurements of a larger number of proper motions. These will be particularly useful in discriminating between genuine and false pulsar-supernova associations and can provide independent measurements of pulsar ages.

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Energy spectrum of ultra high energy cosmic rays

At energies above $\sim 10^{17} \text{ eV}$ it becomes increasingly difficult to maintain the observed near isotropy of ultra high energy cosmic rays if they are of Galactic origin, unless there is an extensive halo magnetic field, and the primaries are heavy nuclei¹; instead, extragalactic origin is often assumed. The models proposed can be divided into three main groups: (1) 'universal', in which there is a class of galaxies in the Universe (of which our own is not a member) which are roughly uniformly distributed, and which generate the ultra high energy particles; (2) 'universal plus supercluster enhancement'², in which the existence of a supercluster is assumed—the enhanced density of galaxies in the SC allows a larger fraction, than is its share by volume, to have come from this region; and (3) 'Supercluster trapping'³, where energetic phenomena in the SC generate the particles which are then trapped for periods of perhaps 10^9 – 10^{10} yr.

The problem with the universal model is that the universal black body radiation (2.7 K) should cause a rapid cutoff in primary intensity above about $6 \times 10^{19} \text{ eV}$ because of photo-meson production^{4,5}, whereas the measured primary spectrum

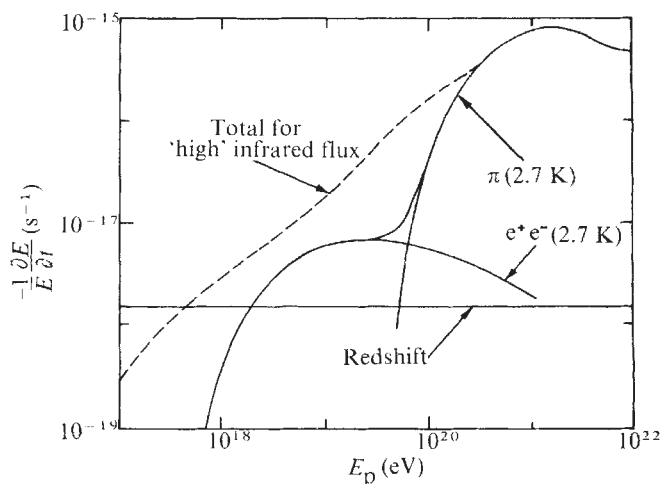


Fig. 1 Fractional rate of energy loss of protons. The solid lines represent the losses from π and e^+e^- -production on the 2.7 K radiation field². The dashed line is the aggregate rate of energy loss for the radiation field described in the text.

extends to 10^{20} eV (refs 6, 7 and papers in ref. 8), and perhaps to $3 \times 10^{20} \text{ eV}$, without any indication of steepening. A similar situation exists in the 'supercluster trapping' model if the lifetime approaches the Hubble time.

We postulated the 'supercluster enhancement' model² in an effort to circumvent the difficulty by deriving quite a large fraction of the highest energy particle from nearby sources. There are difficulties in such a model, however, as we pointed out, because of the need to assume near-straight line trajectories; furthermore, there are questions about our membership of a supercluster (B. T. Jones, unpublished; J. N. Bahcall and P. C. Joss, unpublished).

The possibility of the extragalactic particles commencing their lives as heavy nuclei has been examined⁹⁻¹². The conclusion is that heavy nuclei are not very helpful, but an important feature that has arisen from the examination of the propagation of heavy nuclei concerns the magnitude of the infrared component in extragalactic space. Puget and Stecker¹² derived so-called high and low infrared spectra. The 'high' infrared spectrum is quoted as $n(\epsilon) = 1.1 \times 10^{-3} \epsilon^{-2.5} (\text{cm}^{-3} \text{ eV}^{-1})$ valid for $2 \times 10^{-3} \text{ eV} < \epsilon < 0.8 \text{ eV}$ (giving a total energy density of $\sim 5 \times 10^{-2} \text{ eV cm}^{-3}$). There is no question of this being a definitive spectrum, in view of the paucity of observational data, but at least it is a possibility.

Our contribution now is to revert to the earlier situation⁹ where extragalactic protons were considered. Figure 1 shows the fractional rate of energy loss for the case where only the 2.7 K radiation is considered and its modification when the 'high' infrared spectrum is included. It is now seen that the rapid rise in $(-1/E) (dE/dt)$ at $\sim 6 \times 10^{19} \text{ eV}$ disappears, and instead there is a rather smooth variation over the whole range 5×10^{17} – 10^{21} eV . The slope of the fractional rate of energy loss as a function of primary energy is almost unity, and this means that, for the universal model, if extragalactic sources have a production spectrum of the form E^{-2} then, above $\sim 5 \times 10^{17} \text{ eV}$, the spectrum at the earth will be close to that measured. (In this model the majority of particles below $\sim 5 \times 10^{17} \text{ eV}$ are of galactic origin.) The assumption that extragalactic sources may have flatter spectra than that pertaining to our own Galaxy (for which the differential exponent is probably ~ 2.6 : the measured exponent below $\sim 10^{15} \text{ eV}$), is not unreasonable since these sources are more energetic.

For the supercluster enhancement model, there would again be agreement between observation and expectation; in fact there would be a flattening of the spectrum above about 10^{19} eV , where the SC contribution would start to dominate. It is pertinent to point out that there is indeed some evidence favouring such a flattening¹³, a result that is also, incidentally,

reasonable for a universal model in which sources having a variety of exponents contribute. Finally, the supercluster trapping model would give a very similar result to that for the universal model, if the particle lifetime is $\sim 10^{10}$ yr. For a shorter lifetime the agreement would be reduced because the modulation caused by passage through the radiation fields would be smaller, although the infrared flux density within a supercluster will be higher than the universal average and some measure of compensation might be expected.

If the arguments advanced against the Galaxy belonging to the supercluster are correct and there is, in consequence, neither enhancement nor trapping, then the present suggestion seems to us to be one of the few ways of keeping a model with extragalactic protons. If later measurements show that the adopted infrared intensities are overestimates, then it is more likely that the ultra high energy particles are heavy nuclei of galactic origin and that there is an extensive halo.

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Solar flare effects in equatorial counter electrojet currents

THE solar flare effect on the horizontal component of the geomagnetic field (H) during the period of counter equatorial electrojet current is characterised by a negative crochet (decrease of the H field) at an equatorial electrojet station and a positive crochet (increase of H field) at a low latitude station outside the electrojet belt. During the period of partial counter electrojet, the crochet in H may be positive at all low latitude stations but the amplitude decreases at stations nearer to the equator. We explain this as the combined effect of the solar flare on the normal eastward currents over the Equator associated with Sq current system (around 107–110 km) as well as on the westward counter electrojet current (~ 100 km) both flowing simultaneously over the low latitude stations, while at stations outside the equatorial region the solar flare effect is due to only the eastward Sq currents. (The Sq currents are currents in the upper atmosphere arising from convective motion through the geomagnetic field^{1,2}.)

McNish³ has shown that disturbances in the geomagnetic field during a solar flare (crochet effect) were similar in direction and magnitude to the normal Sq vectors, suggesting that the crochets arise because of the intensification of the Sq current from additional ionisation by the radiations from the solar eruptions. Subsequent analyses, however, showed systematic differences between the current patterns of the crochet and the Sq field⁴. These differences led to the idea

that normal Sq currents are restricted mainly to the E-region, but during the period of solar flare the ionospheric currents extend down to the D-region of the ionosphere.

Nagata⁵ showed that the magnitude of the crochet in the H field is abnormally large at Huancayo and at other low-latitude stations. Forbush and Casaverde⁶ showed that the amplitude of crochets in H at Peruvian stations varied with latitude in a manner similar to the Sq(H) range. At times the

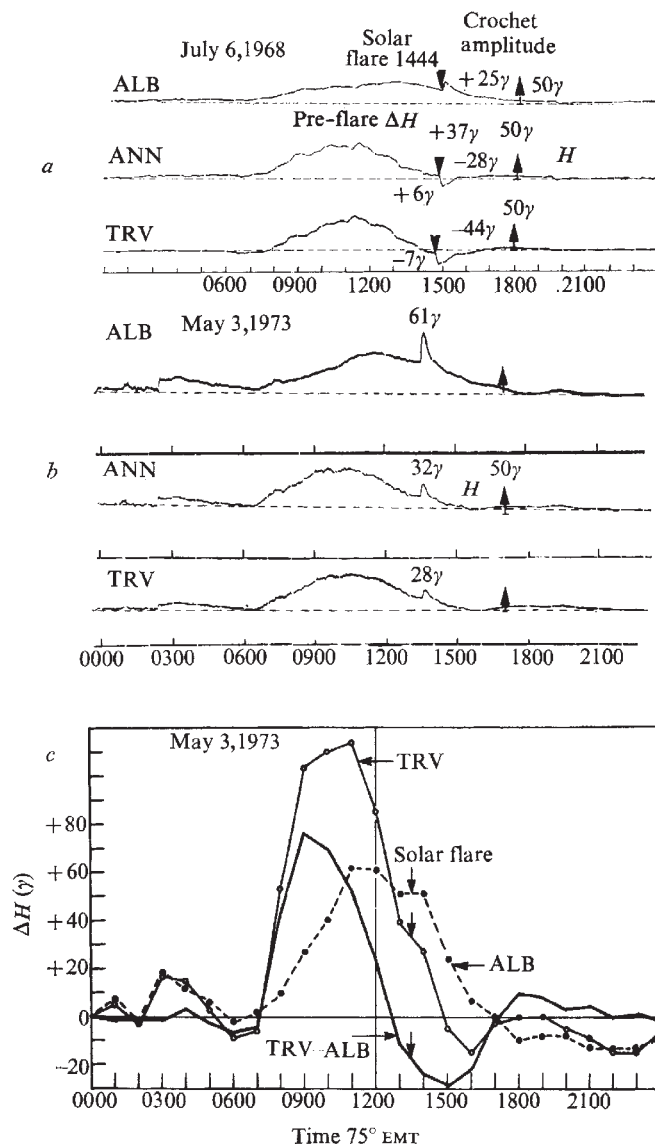


Fig. 1 a, Crochet effect in H at Indian stations during a complete counter-electrojet event on July 6, 1968 at Trivandrum (TRV), Annamalainagar (ANN), and Alibag (ALB). b, Crochet effect in H at Indian stations during a partial counter-electrojet event on May 3, 1973. c, Diurnal variations of the geomagnetic H field at TRV, ALB and TRV-ALB on May 3, 1973.

electrojet currents over a low-latitude station during the day-time are found to be reversed, that is, the currents flow westward causing a depression in the H field. These events called counter-electrojet are closely associated with the various phenomena on the lower as well as the upper ionosphere⁷. Rastogi⁸ postulated the simultaneous existence of Sq currents at altitudes of ~ 107 km over the equatorial zone always flowing eastward and other currents at ~ 100 km flowing either westward or eastward. The net change in the geomagnetic H field at ground level is produced by the effects of both the current systems. If the solar flare somehow increases the

existing currents in the ionosphere, the effects of solar flares during periods of counter equatorial electrojets should be interesting. Here we examine magnetograms from Trivandrum (dip 0.6° S), Annamalainagar (dip 6° N) and Alibag (dip 25° N) for 1958–1973.

Figure 1a and b show magnetograms from these observatories during solar flares on July 6, 1968 at 1444 LT, when there was a clear counter electrojet, and on May 3, 1973 at 1330 LT, when there was a partial counter electrojet. Figure 1c shows the variations of the H field at Trivandrum, Alibag and the difference of the field between the two stations.

On July 6, 1968 the field at Trivandrum was below the night level when the flare occurred. The pre-flare ΔH was -7γ at Trivandrum, $+6\gamma$ at Annamalainagar and $+37\gamma$ at Alibag. The solar flare produced a change of -44γ at Trivandrum, -28γ at Annamalainagar and $+25\gamma$ at Alibag. Thus the crochet in H had opposite signs at stations within the electrojet belt and outside it, if the flare there was a counter electrojet.

For the solar flare on May 3, 1973, Srivastava⁹ noted that the magnitude of crochet in H was larger at Hyderabad (dip 21° N) than at the equatorial stations. The magnitude of ΔH due to the solar flare was 28γ at Trivandrum, 32γ at Annamalainagar and 61γ at Alibag. Thus the crochet amplitude in H increased with increasing distance from the Equator, as opposed to the nature of the latitudinal variation Equatorial stations¹⁰.

Figure 1c shows that after sunrise, H increased faster at Trivandrum than at Alibag until about noon. After 1230 LT, ΔH was smaller at Trivandrum than at Alibag. The ΔH (TRV—ALB) was negative from about 1230 to 1700. It has been shown that when ΔH at an equatorial station is positive, but the difference between this and ΔH at a station outside the electrojet region is negative, then the reversal of ionospheric drifts as well as the disappearance of plasma irregularities at the base of the ionospheric E region indicate a westward electric field, and so a counter-electrojet current is inferred⁸. Here, the q type of sporadic E at Kodaikanal had disappeared on the ionogram at 1245 LT precisely when ΔH (TRV—ALB) had reduced to zero and about half an hour before the occurrence of the flare. These facts clearly indicate the existence of the westward currents at the base of the E -region at the time of the occurrence of crochet on May 3, 1973.

At the stations outside the equatorial electrojet currents, Hyderabad and Alibag, where only the normal Sq currents exist, the crochet in H was due to the increase in the Sq currents only. At the equatorial stations, Trivandrum, Kodaikanal and Annamalainagar, the crochet in H was due to the combined effects of the intensifications of the normal Sq currents as well as of the counter-electrojet currents at a slightly lower altitude. Thus the magnitude of crochet in H on May 3, 1973 increased with distance of the stations from the Equator.

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Accretional capture of the Moon

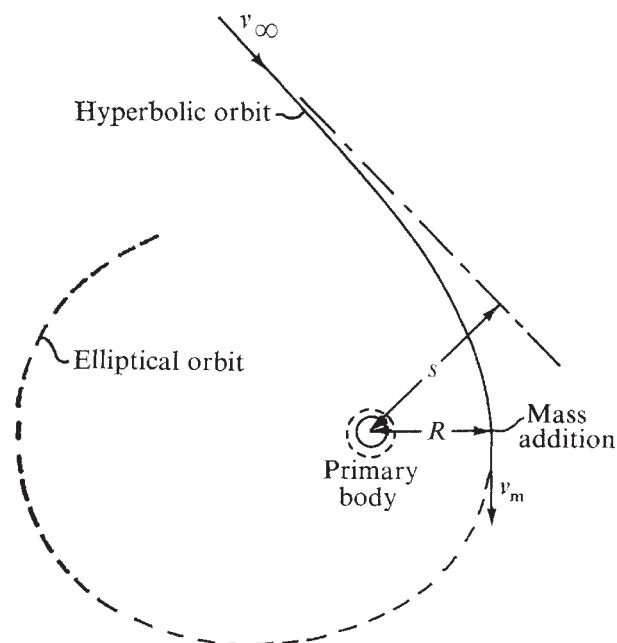
HYPOTHESES for the formation of the Earth–Moon system fall into one of three categories. The Moon was either (1) formed by fission from the Earth, (2) formed by accretion in Earth orbit, or (3) formed in solar orbit and then captured. We suggest that hypotheses (2) and (3) may be combined, with the result that many of the constraints on lunar origin are satisfied.

The primary difficulty with the hypothesis of lunar capture is its inherent improbability. Either collision or escape is much more likely, but capture could take place if sufficient dissipation occurred during a close approach of the Earth and Moon. It is unlikely, however, that tidal dissipation would be sufficient; an alternative would be collisional interactions with material already in Earth orbit¹. Capture could also occur because of a change of mass of the Earth or Moon during a close approach². It is this mechanism which we will discuss here. The closely related problem of orbital behaviour during mass loss from a binary star system has been considered by a number of authors^{3–5}.

Many calculations of planetary accretion have been carried out using particle mechanics. These calculations show that when an accreting body reaches a critical size, the gravitational attraction of the body greatly increases the rate of accretion. Opik⁶ showed that if the Earth and Moon were accreted from common source material, and if the Earth was only slightly larger than the Moon during the early stages of accretion, the preferential gravitational accretion of the larger body could lead to the present mass difference. One consequence of this hypothesis is that the Moon should be enriched in early condensates; this is in fact the case^{7,8}. We propose that capture took place during the final stages of the accretional process.

To estimate the effectiveness of accretional capture, we consider the following simple model: a small secondary body of mass m , in a hyperbolic orbit, approaches a large primary body of mass M . When the separation is a minimum, the mass of the primary body is increased to $M+\Delta M$. We derive the conditions under which this change in mass will result in an elliptical orbit (capture). The process is illustrated in Fig. 1.

Fig. 1 Illustration of accretional capture: the transfer from an initial hyperbolic orbit to an elliptical orbit due to mass addition of mass to the primary body at the point of closest approach.



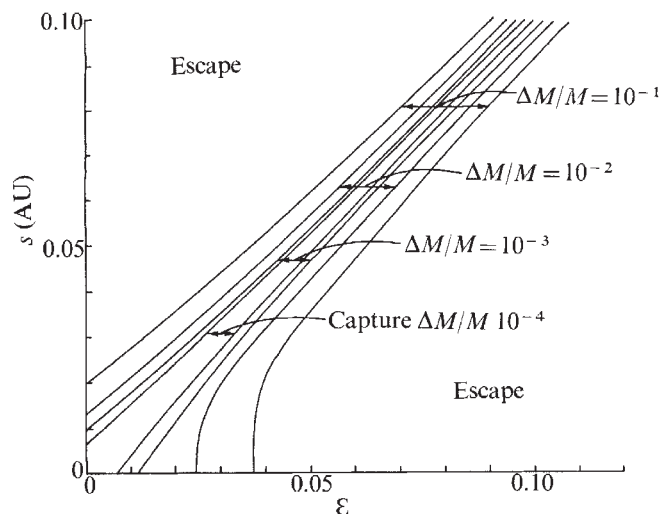


Fig. 2 Capture window for accretional capture: required values for the minimum separation s and eccentricity ϵ of the lunar orbit for various fractional increases in the Earth mass at the point of closest approach.

The minimum radial separation R , and the corresponding maximum relative velocity v_m are related to the approach velocity v_∞ and the impact parameter s by

$$\frac{s}{R} = \frac{v_m}{v_\infty} = \frac{GM}{s v_\infty^2} + \left(1 + \frac{G^2 M^2}{s^2 v_\infty^4}\right)^{1/2} \quad (1)$$

The sudden increase in the mass of the primary body is a non-conservative process, since there is a discontinuous change in the potential energy of the system. The secondary body suddenly finds itself in a stronger gravitational field, and may not have enough potential energy to escape.

After the change of mass, the total energy of the system is

$$E = \frac{1}{2} m v_m^2 - \frac{m(M + \Delta M)G}{R} \quad (2)$$

Capture occurs when $E < 0$; the threshold value for capture is $E = 0$. Combining (1) and (2) with the condition $E = 0$ gives the mass increase required for capture

$$\Delta M = \frac{M}{2} \left[\left(1 + \frac{s^2 v_\infty^4}{M^2 G^2}\right)^{1/2} - 1 \right] \quad (3)$$

In order to relate the impact parameter s to the approach velocity v_∞ , we assume that the two bodies are in independent orbits about the Sun. The primary body (the Earth) is assumed to be in a circular orbit with a radius r_e of 1 AU; the secondary body (the Moon) is assumed to be in an elliptic orbit with an eccentricity ϵ . The impact parameter s is taken to be the minimum radial separation between the bodies, and v_∞ the corresponding difference in orbital velocities; assuming $s/r_e \ll 1$ and $\epsilon \sim 1$ these are related by

$$v_\infty = \pm \frac{1}{2} \left(\frac{GM_\odot}{r_e} \right)^{1/2} \left(\frac{s}{r_e} - \epsilon \right) \quad (4)$$

Note that if $\epsilon \approx s/r_e$, the approach velocity is small and the probability of capture is enhanced.

Combining equations (3) and (4) gives

$$\frac{s}{r_e} - \epsilon = \left(64 \frac{M \Delta M r_e^2}{M_\odot^2 s^2} \right)^{1/4} \quad (5)$$

Taking M to be the present mass of the Earth, the necessary conditions for capture are given in Fig. 2 for various values of $\Delta M/M$. It is seen that there is a well defined capture window even for small mass increments. This capture window corresponds to the condition of low relative velocity when $\epsilon \approx s/r_e$.

Clearly the above analysis is highly idealised. The influences of a finite rate of mass addition and of the gravitational attraction of the Sun should be considered. The analysis does, however, indicate the possible importance of accretional capture, and indicates that further studies should be carried out.

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Petrogenesis of eucrite, howardite and diogenite meteorites

THE mineralogical, textural and compositional characteristics of the eucrite meteorites suggest that they are the products of igneous processes. Howardites are polymict breccias containing a variety of fragments, the bulk of which are thought to have crystallised from eucrite and eucrite-related melts¹. Diogenites are orthopyroxene-rich stones which may represent cumulates from eucrite-related melts². I relate here experimentally determined phase equilibria to the igneous processes which produced the eucrites and their associates.

I conducted melting experiments on a sample of the Juvinas eucrite (Harvard 87) at 1 atm total pressure and various oxygen fugacities. Samples were held in iron or molybdenum foil capsules in quenching furnaces with oxygen fugacity controlled by H_2 - CO_2 gas mixtures³. Phase compositions were determined with an electron microprobe. At $f_{O_2} = 10^{-13.4}$ atmospheres and less than 10 °C below the metal-saturated olivine liquidus (Fo_{67} ; 1,183 °C), the sample is saturated with olivine (Fo_{65}), pigeonite ($Wo_{55}En_{45}$), plagioclase (An_{94}), chrome-rich spinel and iron metal. On cooling, olivine reacts out by 1,150 °C and a silica polymorph crystallises from 1,085 °C.

Figure 1 is a projection of a low pressure liquidus diagram relevant to eucrite crystallisation. Olivine is in reaction relation with liquids at peritectic A (Fig. 1), and on the olivine + pyroxene boundary curve. Eucrite compositions have been projected onto this diagram. The clustering of non-cumulate eucrite compositions about the olivine + low calcium pyroxene + plagioclase + chrome spinel + metal peritectic (A , Fig. 1) indicates that like Juvinas, these other eucrites are probably multiply saturated near their liquidus. This emphasises the role of low pressure crystal-liquid equilibria in the genesis of the eucrites: all known eucrites can be designated as either multiply saturated liquids at A (Fig. 1) or cumulates or partial cumulates of the pyroxene and plagioclase near the liquidus of such liquids. Any model of the igneous processes which produced the eucrites must account for this preferential generation of liquids at peritectic A (Fig. 1).

The compositions of pyroxenes produced in the Juvinas experiments are similar to the compositions of pyroxenes in

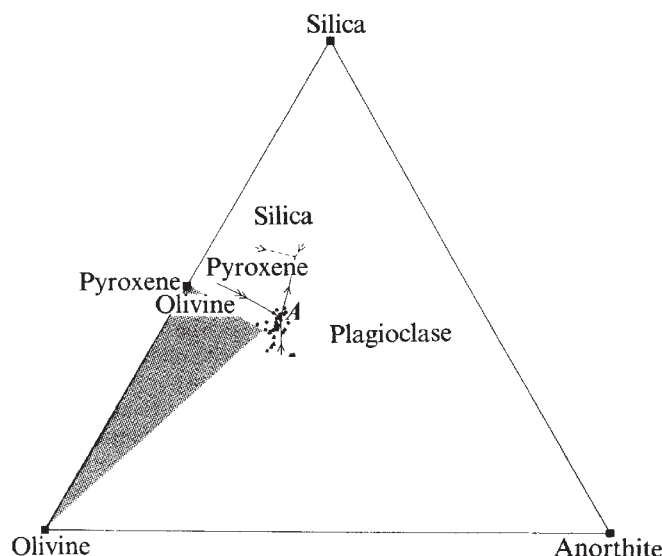


Fig. 1 Projection of low pressure liquidus diagram valid for molar $\text{Fe}/\text{Fe} + \text{Mg} \approx 0.60$. Field boundaries were located by microprobe analyses of multiply saturated liquids from experiments on the Juvinas eucrite. Iron metal and chrome-rich spinel are present in all fields. $\blacktriangle$, Projected analyses of cumulate eucrites: Moore County (2 analyses), Serra de Magé (2 analyses), Moama, Medaniitos; $\bullet$, Projected analyses of other eucrites. Pre-1960 analyses are plotted only when a more recent analysis of the same stone is not available. Analyses are plotted on this diagram by recalculating SiO_2 , Al_2O_3 , FeO , MgO , CaO , Na_2O and K_2O in terms of molar SiO_2 , $\text{CaAl}_2\text{Si}_2\text{O}_8$, Fe_2SiO_4 , Mg_2SiO_4 , CaSiO_3 , $\text{NaAlSi}_3\text{O}_8$ and KAlSi_3O_8 and then projecting on to the olivine-anorthite-silica plane. Analyses which plot below the pyroxene-plagioclase join do so because of the effects of modal iron metal, troilite, chromite, ilmenite and calcite on the projection. *A* and the shaded region, are explained in the text.

cumulate and non-cumulate textured eucrites and to those of many pyroxenes in howardites, but are more Fe-rich than the pyroxenes and olivines in diogenites and the most magnesian pyroxenes and olivines in howardites. These natural Mg-rich phases would have equilibrated with liquids more magnesian than the eucrite magmas at *A* (Fig. 1), and any model of eucrite petrogenesis should account for the existence of such liquids.

Reduction of eucrite magma resulting in crystallisation of iron metal and a decrease in the Fe/Mg and Fe/Mn of the magma could produce magmas which would crystallise magnesian diogenitic and howarditic pyroxenes. The pyroxenes crystallised from such liquids would have a higher Mn/Fe than those crystallised from unreduced eucrite magmas. Such an origin for diogenitic and the most magnesian howarditic pyroxenes is, however, unlikely since the Mn/Fe ratios of these phases are nearly identical to those of pyroxenes in cumulate and non-cumulate eucrites and of the more iron-rich howarditic pyroxenes^{2,4}.

Models which produce eucrite liquids by the differentiation of magnesian parental magmas and which invoke the fractional crystallisation of eucrite magmas to explain the bulk of the chemical variation observed in eucrites² encounter several difficulties. First, as pointed out in connection with Fra Mauro rocks and soils, which display a similar clustering about an olivine + plagioclase + low calcium pyroxene peritectic, it is difficult to preferentially generate liquids at peritectic *A* (Fig. 1) by low pressure differentiation of other magmas through the action of crystallisation processes⁵. Second, if the proposed parental magmas ever passed through a stage in their evolution in which pyroxene was the only crystallising phase (required by such models to produce the diogenites and magnesian pyroxenes in howardites), the liquid lines of descent of such magmas would never encounter the peritectic at *A* (Fig. 1). Third, low

pressure fractional crystallisation paths which can produce liquids at *A* (Fig. 1) involve the crystallisation of plagioclase and olivine, but not of pyroxene. At low oxygen fugacities, the fractionation of plagioclase would rapidly differentiate europium from the other rare earth elements. Models which attempt to derive magmas at peritectic *A* (Fig. 1) by the low pressure fractional crystallisation of more magnesian magmas are, therefore, not easily reconciled with the primitive rare earth distributions of some eucrites⁶, the existence of diogenitic and howarditic pyroxenes more magnesian than the pyroxenes at the liquidus of magmas at *A* (Fig. 1), or the rarity of olivine in diogenites, howardites and eucrites. Fourth, these models cannot easily reconcile the constancy of the major element chemistry (particularly the $\text{Fe}/\text{Fe} + \text{Mg}$ ratios) of eucrites which cluster at *A* (Fig. 1) with their variations in rare earth and incompatible element concentrations⁶. Though the variations in the concentrations of rare earths and incompatible elements in the eucrites at *A* (Fig. 1) apparently suggest that these eucrites could be related to each other through the fractional crystallisation of up to 60% of a liquid of the composition of Haraiya² (another eucrite which projects at *A* (Fig. 1)), that suggestion is incompatible with the fact that all of these eucrites have nearly identical major element chemistries. A liquid which has reached *A* (Fig. 1) will not remain there during fractional crystallisation; as it loses heat it will change composition along the pyroxene + plagioclase cotectic and trend toward iron enrichment. Fifth, if these models are valid, the total volume of the magnesian parental magma must have greatly exceeded the volume of eucrite magma. Examples of magmas more primitive than the eucrites should predominate among falls and the distribution of fragments in howardites should be strongly skewed toward the products of magmas more magnesian than the eucrites at peritectic *A* (Fig. 1). These relationships are not observed.

I propose the following model to account for the observed features of the eucrites and their associates. The eucrite magmas at the peritectic *A* (Fig. 1) are interpreted as the products of small degrees of low pressure partial melting of an olivine + low calcium pyroxene + plagioclase + chrome spinel + iron metal source which projects in the shaded region of Fig. 1. Compositional variation of the eucrites is the result of variable degrees of partial melting and subsequent fractional crystallisation and crystal accumulation. Diogenites are regarded as cumulates of phases separated from more advanced partial melts of the same source. Howardites are polymict breccias containing fragments sampling the spectrum of differentiated liquids and cumulates developed from the range of primary magmas produced by variable degrees of partial melting of this source.

Eucrite magmas produced by the partial melting of such a source would plot at the peritectic *A* (Fig. 1) until plagioclase was exhausted from the source region; this explains the observed clustering of eucrite compositions. If the eucrites represent small degrees of partial melting, variations in the degrees of partial melting which produced different eucrite liquids could have produced the significant variations in the concentrations of incompatible elements which are observed between eucrites of indistinguishable major element chemistry and $\text{Fe}/\text{Fe} + \text{Mg}$ ratios⁶. If plagioclase becomes exhausted from the source region at small degrees of partial melting, this model can also explain the variations in rare earth distributions and the magnitudes of europium anomalies which are observed between eucrites of nearly identical major element chemistry⁶.

The subsequent crystallisation and separation of pyroxene and plagioclase from the primary magmas at *A* (Fig. 1) would be expected to produce cumulates and silica and incompatible element enriched liquids of higher $\text{Fe}/\text{Fe} + \text{Mg}$ ratios projecting near the pyroxene + plagioclase cotectic emanating from *A* (Fig. 1). Moore County and Serra de Magé are examples of these cumulates¹. The bulk chemistry and mineralogy of Binda, considered by some to represent a magma parental to the eucrites², are compatible with such a cumulate origin. Nuevo Laredo and

Lakangaon may represent liquid differentiates from the crystallisation of magmas at *A* (Fig. 1). Both are enriched in incompatible elements and display the iron enrichment expected in differentiated liquids. It is unlikely that they represent smaller degrees of partial melting of the same source that produced the eucrites which are not iron enriched, for at least in the case of Nuevo Laredo, rare earth distributions are not compatible with this interpretation⁶. Other eucrites may have been affected to a lesser extent by fractional crystallisation.

Though olivine would be present in the source rock and the residue left after melting, it would not be present at the liquid of the partial melts at *A* (Fig. 1), (provided there was little fall in pressure accompanying eruption) because it is in reaction relation with these liquids. This is consistent with the absence of olivine in eucrites and its rarity in howardites.

If the reaction relationship between iron metal and silicate liquids observed in synthetic systems⁷ extends to this natural system, metal would remain in the source region until all crystalline silicate phases were removed by melting. The probable reaction relation between chrome-rich spinel, low calcium pyroxene and liquid⁸ suggests that chrome spinel would remain in the source region until all the pyroxene had been removed by melting.

In conditions of fractional fusion⁹, the production of magma by further melting would be nearly halted after the exhaustion of plagioclase from a source projecting in the shaded region of Fig. 1. If equilibrium fusion or imperfect fractional fusion took place, the melts produced after the exhaustion of plagioclase from the source would move up the olivine + pyroxene curve (Fig. 1), continuously decreasing the Fe/Fe+Mg ratio, until pyroxene was exhausted from the source. The diogenites and magnesian pyroxenes in howardites can be interpreted as cumulates of the pyroxene crystallising near the liquid of these more advanced partial melts. The rarity of olivine in these meteorites is predicted since these liquids would not ordinarily crystallise olivine. The occasional presence of olivine in these meteorites (and Chassigny) could result from variations in source composition, variations in conditions of melting or crystallisation (for example, a small drop in pressure between the source region and the environment of crystallisation), or xenocrysts from the imperfect separation of the magma from the source. The absence of cumulate plagioclase in the diogenites or in intergrowths with the most magnesian howarditic pyroxenes⁴ can be expected since plagioclase would not be present at the liquid of these magmas.

The residue left in the source region after the exhaustion of plagioclase by partial melting would be olivine + low calcium pyroxene + chrome spinel + metal. If metal were a major constituent of the source, this residue would approximate the Lodran meteorite¹⁰. If the source were low in metal, the olivine-bearing diogenites could represent such residues. Since such an origin is not possible for the olivine-free diogenites, a cumulate origin is preferred for the entire diogenite group.

Further equilibrium fusion or imperfect fractional fusion of this same source after the exhaustion of pyroxene from the source region would produce liquids moving away from the olivine + pyroxene reaction curve (Fig. 1) towards the olivine composition, leaving a pallasite-like residue of olivine + chrome spinel + metal. The olivines crystallised near the liquid of such melts may account for some howarditic and diogenitic olivines.

The abundance patterns of fragments in howardites⁴ and the lack of known meteorites with compositions which could represent partial melts more advanced than the eucrites at *A* (Fig. 1) are consistent with the bias toward the products of smaller degrees of partial melting which is expected in conditions of imperfect fractional fusion.

Though eucrite liquids would have equilibrated with the metal of the residue left in the source region after partial melting, metal would not be at the low pressure liquid of these melts because of the probable reaction relation between metal and silicate liquid (provided no change in oxidation state of the

magmas occurred between the source region and the environment of crystallisation). This is compatible with both the observed low Ni contents of the eucrites, which suggest they have equilibrated with metal, and the petrographic observations that metal is never found as a cumulate phase in diogenites and cumulate eucrites, is rarely enclosed in pyroxenes, and is usually a late, interstitial phase when it occurs¹¹. The late stage crystallisation of metal in eucrites could be attributable to desulphurisation processes similar to those which have been proposed to account for metal in lunar mare basalts¹².

A partial melting model requires a suitable low pressure source assemblage in the eucrite parent body. A primitive type of mesosiderite (perhaps a condensate¹³ or the product of early differentiation and brecciation on the eucrite planet or on some other body which was subsequently incorporated into the eucrite planet) could be suitable source material. The low pressure mineralogy (< 8 kbar) of the bulk composition of the eucrite parent body calculated by Wänke and Palme¹⁴ is similar to the source assemblage required by this model. This similarity raises the possibility that the eucrites may have formed by partial melting of the primitive material of the eucrite parent body at 4.6×10^9 yr BP.

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Does metallic ammonium exist?

THE ammonium ion NH_4^+ behaves in many ways like an alkali metal ion. This led Ramsey¹ to propose that monovalent ammonium metal (NH_4^+ ions immersed in an almost uniform, degenerate sea of electrons) is stable at pressures much lower than typical insulator–metal transition pressures² of megabars $\sim 10^{11}$ Pa (10^5 Pa = 1 bar). Subsequently, it was estimated³ that the transition from a mixture of NH_3 and H_2 molecules to metallic ammonium, occurs at a pressure of less than 2.5×10^{10} Pa. If this estimate were correct, metallic ammonium would be of great interest to experimental high pressure physicists, and to planetary physicists. Indeed, metallic ammonium has been invoked in some^{4,5} but not all models for the interiors of Uranus and Neptune; and its existence could be of importance⁶ for calculations of the interior magnetic fields of these planets.

Here I report new calculations which demonstrate, rather conclusively, that metallic ammonium, at least in the form envisaged by Ramsey, is unstable at all pressures. My calculations disagree with those of ref. 3 in two important respects: first, I find that the insulating $\text{NH}_3\text{--}\frac{1}{2}\text{H}_2$ mixture is sufficiently compressible to remain the favoured phase, at least until pressures of $\sim 10^{11}$ Pa, even if the metallic phase calculation of ref. 3 is correct. Second, my calculation for the metallic phase gives a systematically higher energy than was obtained in ref. 3. These results are summarised in Fig. 1.

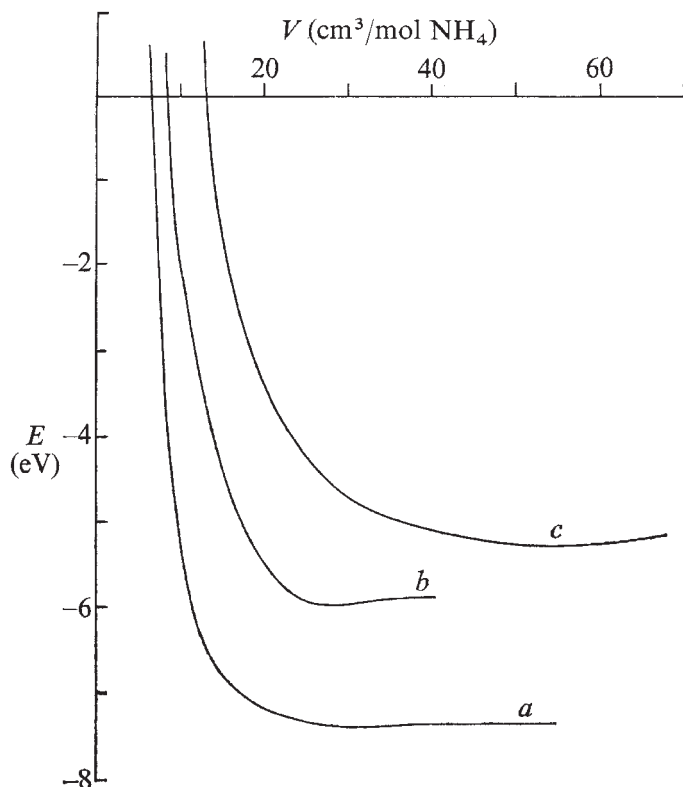


Fig. 1 A comparison of the energy-volume relationships for molecular $\text{NH}_3\text{-}\frac{1}{2}\text{H}_2$ (a) and metallic ammonium (b, ref. 3; c, my results).

The equation of state for the $\text{NH}_3\text{-}\frac{1}{2}\text{H}_2$ mixture was calculated assuming volume additivity, so that $V(P)$, the specific volume of the mixture at pressure P , is given by

$$V(P) = V(\text{NH}_3, P) + \frac{1}{2}V(\text{H}_2, P) \quad (1)$$

where $V(\text{NH}_3, P)$ and $V(\text{H}_2, P)$ are the molecular volume-pressure relations of ammonia and hydrogen solids, respectively. The equation of state for ammonia is known at low pressures ($P < 2 \times 10^9$ Pa) from experiment⁷, and at very high pressures ($P \gtrsim 2 \times 10^{11}$ Pa) from Thomas-Fermi-Dirac theory⁸. The intermediate region is estimated by interpolation, but the uncertainties in volume do not exceed $\sim 15\%$. The equation of state for hydrogen is taken from Ross's analysis⁹ of the Livermore shock data. From $V(P)$, the energy of the mixture is evaluated:

$$E(P) - E(0) = - \int_0^P P' \frac{dV(P')}{dP'} dP' \quad (2)$$

at $T = 0^\circ\text{K}$. In Fig. 1, the energy zero is chosen to be the energy of infinitely dispersed NH_4^+ ions and electrons. Thus,

$$E(0) = -(\frac{1}{2}D + I_H - A_p + B) \quad (3)$$

where $D = 4.44$ eV is the dissociation energy of the hydrogen molecule, $I_H = 13.54$ eV is the ionisation energy of the hydrogen atom, A_p is the proton affinity of the ammonia molecule, and $B \sim 0.1$ or 0.2 eV is the binding energy of the $\text{NH}_3\text{-}\frac{1}{2}\text{H}_2$ crystal mixture. The most likely value of A_p is 8.6 eV (ref. 10), but the error in this figure could be as much as 0.3 eV. This gives $E(0) = -7.3$ eV. The resulting energy-volume relation is shown in Fig. 1. Even allowing for the uncertainties in A_p and $V(P)$, the curve never crosses the metallic phase curve of ref. 3. Their transition pressure estimate is invalid since they did not calculate the $\text{NH}_3\text{-}\frac{1}{2}\text{H}_2$ equation of state, but merely constructed

that tangent to the metal-phase curve which passes through the molecular-phase zero pressure point.

It must be emphasised that my conclusion is not contingent on the validity of volume additivity (equation (1)). My calculation is correct for a conceivable state: a mixture of macroscopic crystals of pure NH_3 and H_2 . If an alloy is preferentially formed it can only have lower energy than the calculation predicts—this would make the molecular phase even more favourable.

Since the NH_4^+ ion is about the same size as the Rb^+ ion, simple pseudopotential theory¹¹ would lead us to expect that the equilibrium ($P = 0$) volume per ion of metallic ammonium is comparable with that of rubidium. The detailed calculations confirm this prediction of the simple (but very successful) pseudopotential theory. In contrast, an equilibrium volume was found in ref. 3 that was about a factor of two smaller, and more comparable to metallic sodium. My calculation is based on the Wigner-Seitz approach, but with the potential given by the self-consistent density-functional theory of Kohn and Sham¹², which includes all exchange and correlation effects in a local but self-consistent approximation. The Bardeen perturbation theory¹³ was used to evaluate the effective (band) mass of the conduction electrons.

The method was tested on metallic sodium, and the results were -6.38 eV for the total energy per ion, and $3.96a_0$ for the radius of the Wigner-Seitz sphere (where $a_0 = 1$ Bohr $= 0.529 \times 10^{-8}$ cm); these are in excellent agreement with the observed values¹² of -6.27 eV and $3.93a_0$, respectively. For metallic ammonium, I used the spherical potential of ref. 3 and also their core wavefunctions. The results were -5.40 eV for the energy and $5.18a_0$ for the radius, whereas they obtained -5.84 eV and $4.23a_0$, respectively. I also made a calculation using more accurate core wavefunctions¹⁴ and a correspondingly larger N-H bond length ($1.91a_0$ instead of $1.84a_0$), obtaining -5.36 eV and $5.35a_0$ for $P = 0$. The corresponding energy-volume relation is the upper curve in Fig. 1. At high pressure, the energy probably rises even more rapidly than shown, because of the Born-Mayer repulsion between the ion cores. Corrections for the non-spherical symmetry of the NH_4^+ ion have been estimated and are negligible at low pressures.

I conclude that monovalent metallic ammonium is never the thermodynamically favoured phase for an $\text{NH}_3\text{-}\frac{1}{2}\text{H}_2$ mixture. Of course, this mixture must eventually become metallic if compressed enough, but this would not occur until pressures of $\sim 10^{11}$ Pa. The very compact metallic phase that would then form is unrelated to the low density metallic phase proposed by Ramsey.

I notice, finally, that a dynamo-generated magnetic field in Uranus or Neptune is not contingent on the existence of metallic ammonium—other sources of electrical conductivity may be adequate (this will be dealt with elsewhere).

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Mercury in the Kuroshio and Oyashio regions and the Japan Sea

ALTHOUGH measurements of the mercury concentration in sea water have been made by several investigators¹⁻¹³, the reported values are widely scattered and it may be significant that almost all the samples were kept in polyethylene bottles after acidification. Weiss *et al.*¹⁴ have reported that the main source of mercury in the oceans was the degassing of the Earth's crust by way of the atmosphere. It should then be supposed that the large experimental variations for open oceans does not reflect the true situation.

One source of error is to be found in the use of polyethylene bottles—we found that nanogram amounts of mercury were

leached out of the plastic by the samples, whose mercury concentrations increased by factors of 2 to 100, even if the bottles had been washed out with nitric or hydrochloric acid before use. The outline has already been reported¹⁵, and the detailed results will be published elsewhere. Recently, sample contamination by polyethylene bottles has been confirmed^{16,17}. In our work, glass bottles and stoppers, which had been checked to be non-contaminating beforehand, were used for sample storage.

Here we report on the mercury concentrations in the Kuroshio and Oyashio regions and the Japan Sea. Samples were collected with Nansen bottles from February to August 1974. Immediately after collection, the water was acidified to 0.4 N with sulphuric acid, and stored in a glass bottle. The samples were analysed at the laboratory after a month's storage.

The outline of the analytical method is as follows¹⁸. Ionic mercury, in a 0.5–0.8-l sample solution, is reduced with stannous chloride, and the mercury vapour released is flushed out with nitrogen on to gold particles packed in a glass tube. The metal is then heated at 500 °C in a furnace, and the vaporised mercury is determined using an atomic absorption spectrophotometer. The error in a single determination was ~ 10% when measuring quantities of ~ 5 ng Hg.

The results are shown in Table 1. Although there were haloclines and thermoclines in the seas at our sampling points, no appreciable change of mercury concentration with depth was found, and the samples showed an almost constant value, 5.0 ± 0.5 ng l⁻¹.

According to Fitzgerald *et al.*¹², if the seawater samples had been stored in plastic bottles, at pH 1, for 10 days, the mercury concentrations would have been 57% lower than those in samples measured immediately, which they therefore did—on board their ship.

We found, however, that for sea water of more than 30‰ salinity, the mercury concentrations were 30% higher than in the samples measured immediately. We believe that the discrepancy arises because of the presence of some mercuric complexes, whose mercury cannot be detected immediately. Acidification to 0.4 N with sulphuric acid for two weeks at room temperature (and longer at lower temperatures) is required to release this mercury, and give the correct total mercury concentration. (For samples with salinities of less than 30‰, the mercury concentration actually decreases, because of adsorption to the glass walls.)

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Table 1 Mercury in the Kuroshio and Oyashio regions and the Japan Sea

Station Date of sampling	Depth (m)	Temperature (°C)	Salinity (‰)	Hg (ng l ⁻¹)
1	0	0.2	32.68	4.5
42° 30' N	20	0.18	32.68	5.6
144° 00' E	49	0.80	32.86	5.6
February 1974	98	0.85	32.94	4.6
	196	1.38	33.16	4.6
2	0	3.00	33.12	5.4
37° 00' N	21	3.52	33.30	3.9
114° 00' E	52	3.53	33.33	4.7
February 1974	101	2.46	33.30	5.5
	200	2.45	33.44	5.5
	500	3.73	34.07	5.6
	1,009	2.90	34.40	5.5
	1,219	2.58	34.46	5.2
3	0	15.6	34.74	5.4
36° 56' N	20	15.81	34.75	5.2
141° 58' E	50	15.78	34.74	5.4
February 1974	101	15.56	34.72	4.9
	197	12.85	34.50	5.4
	486	4.24	33.87	5.4
	782	3.37	34.12	4.9
	1,200	2.65	34.43	5.0
4	0	18.4	34.83	5.2
35° 01' N	21	18.50	34.81	5.4
142° 04' E	52	18.51	34.81	4.5
March 1974	102	18.48	34.80	5.0
	247	16.59	34.69	4.5
	557	8.49	34.22	4.8
	1,036	3.72	34.37	4.9
	1,233	3.13	34.41	5.0
5	0	17.2	34.78	5.0
34° 00' N	50	17.13	34.79	4.0
142° 00' E	102	17.09	34.79	3.6
March 1974				
6	0	28.0	32.73	5.1
33° 11' N	17	26.99	33.39	5.3
127° 32' E	100	15.81	34.43	4.9
August 1974				
7	0	27.9	33.42	5.4
33° 23' N	9	27.58	33.40	5.6
128° 35' E	70	22.39	34.25	5.4
August 1974	94	20.48	34.25	5.4
8	0	22.2	33.50	4.3
38° 41' N	20	18.87	33.72	4.3
135° 55' E	102	13.05	34.44	5.4
July 1974	202	3.57	34.09	4.8
	400	0.56	34.07	4.3
	697	0.19	34.07	4.8
9	0	20.9	33.71	5.0
39° 17' N	20	18.50	33.85	5.2
135° 55' E	50	11.01	34.34	5.0
July 1974	150	2.54	34.08	4.3
	200	1.37	34.06	5.6
	397	0.40	34.02	4.5
	695	0.20	34.01	5.4

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Model for the accumulation of radionuclides in oysters and sediments

THE time variation of the concentration of γ -ray emitting radionuclides has been measured and modelled in a stable population of American oysters (*C. virginica*), and associated estuarine sediments. Radionuclide concentrations were measured for the natural radionuclides ^{212}Pb and ^{40}K and for the man-made radionuclides ^{58}Co , ^{60}Co , ^{54}Mn , ^{95}Nb , ^{134}Cs and ^{137}Cs using a Ge(Li) detector with a massive lead shield and multichannel analyser. Measured concentrations of the most abundant man-made radionuclides observed in this study (^{58}Co , ^{60}Co , and ^{54}Mn) were compared with concentrations calculated with a mathematical model for the accumulation and loss of these radionuclides by oysters and sediments.

Much work has been done in recent years in the accumulation of radionuclides in marine organisms (refs 1 to 4, and references therein). Mathematical models for accumulation and loss of radionuclides are usually based on laboratory studies using a constant source of radionuclides, a condition which is generally convenient. A model for field studies, however, must provide for the time variation of the source of radionuclides, since in the case of nuclear reactors the liquid radionuclide effluent is usually released according to some schedule with various waiting periods between releases, as discussed by Heft *et al.*². This time variation of source intensity can be included in the mathematical model by driving the first order linear differential equation for accumulation and loss with a time varying function representing the release schedule. This model corresponds mathematically to a driven system with characteristic relaxation times for the various mechanisms of accumulation and loss.

American oysters *C. virginica* were reared at four locations at the Montsweag Estuary of the Sheepscot River in southern-central coastal Maine, USA and at a control site six miles east on the Damariscotta River Estuary.

At two month intervals, ~1 kg of live oysters from each site were randomly selected from a larger population of the same age and size. Each sample was removed from its rearing tray, transported to the detector, γ -ray counted in a standard geometry for 5,000 s, and returned to its site. The resulting counts were computer processed using the Compton continuum subtraction method³. The concentrations of radionuclides were determined using standard techniques to calibrate the multichannel analyser-detector-shield system.

Sediments were collected at the same five sites by removing the first centimetre of sediment over an area of sufficient size to provide a 2 kg (wet) sample. Concentrations of radionuclides in the sediments were measured in the same manner as the oysters.

The uptake of radionuclides may be described by a first order linear differential equation

$$dN/dt = -\lambda N + R(t), \quad (1)$$

where dN/dt is the change in atoms of radionuclide, λN is the rate of loss due to radioactive decay, and $R(t)$ is the rate of introduction of radionuclide from an external source (the nuclear reactor release schedule)⁶. Depuration, or biological loss, may be included by writing a 'lambda effective' as the sum of the radioactive decay constant and a biological decay constant.

The solution to equation (1) may be written

$$N = \exp(-\lambda t) \int_0^t R(\tau) \exp(\lambda \tau) d\tau + c \exp(-\lambda t) \quad (2)$$

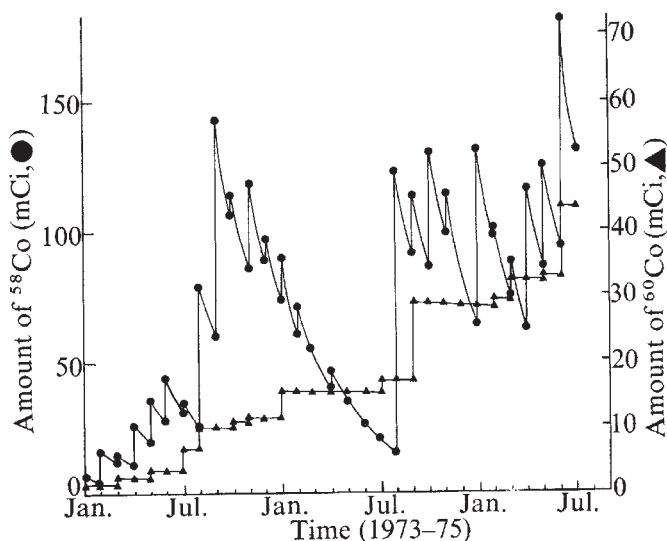


Fig. 1 ●, Theoretical accumulation of ^{58}Co in mCi for the case $U = 1 \text{ g}^{-1}$ plotted against month of year from February 1973 through June 1975. ▲, Theoretical accumulation of ^{60}Co in mCi for the case $U = 1 \text{ g}^{-1}$ against month of the year from February 1973 through June 1975.

We assume that releases of radionuclides are made in a sequence of m times, $\{t_1, t_2, t_3, \dots, t_m\}$ and the amount of nuclide released is given by a function $f(t)$ where

$$f(t) = f_1 \delta(t-t_1) + f_2 \delta(t-t_2) + \dots + f_m \delta(t-t_m)$$

The amount of radionuclide release by the reactor and retained by the sample is given by U , so that, assuming U is a constant, we can construct a solution for the intervals between the times of release.

$$0 \leq t \leq t_1 \quad N(t) = ce^{-\lambda t} \quad (3)$$

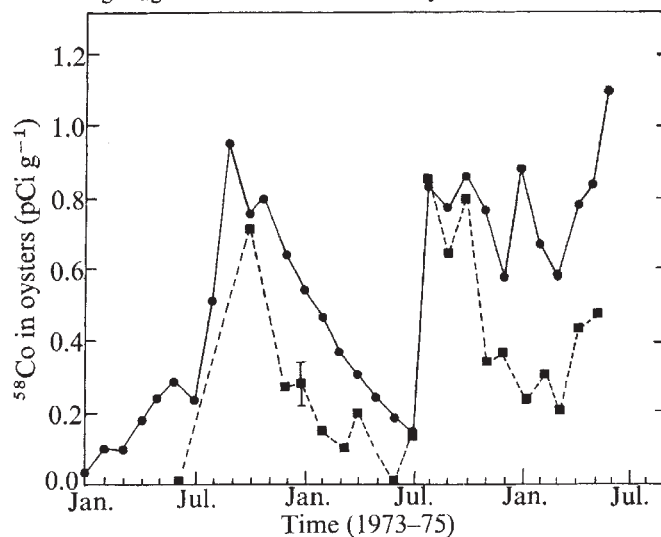
$$t_1 \leq t \leq t_2 \quad N(t) = Uf_1 e^{-\lambda(t-t_1)} + ce^{-\lambda t}$$

$$t_2 \leq t \leq t_3 \quad N(t) = Uf_2 e^{-\lambda(t-t_2)} + Uf_1 e^{-\lambda(t-t_1)} + ce^{-\lambda t}$$

$$\vdots$$

The interpretation of this set of equations is obvious. Sudden increases occur at each time of release. Typically, the radionu-

Fig. 2 ■, Measured ^{58}Co radionuclide concentration in oysters at the reactor outflow site. A typical 1σ error bar is shown. ●, Theoretical curve for ^{58}Co scaled by factor $U = 7.6 \times 10^{-12} \text{ g}^{-1}$ against time in months for the years 1973-1975.



clides are released weekly, over a period of a few hours. For the purposes of illustrating the theory presented here, however, it has been found sufficient, for a bi-monthly sampling schedule, to approximate the actual release schedule with a monthly release in which the total amount of each radionuclide released is assumed to be released instantaneously. Graphs of two typical cases are shown in Fig. 1, which illustrates the results for ^{58}Co (half life 2.4 months) and ^{60}Co (half life 63 months).

The radionuclide concentration for ^{58}Co in oysters expressed in pCi g^{-1} is shown in Fig. 2. The broken line represents the experimental values for this radionuclide at the reactor outflow site and the theoretical results are shown with a solid line. The peaks found in the theory are evident at the reactor outflow as well as the other three Montsweag Estuary sites. In general it is shown that the oysters exhibit a faster decrease in activity than predicted by the radioactive decay constant only. An effective decay constant taking into account the biological decay constant as well can bring the theory into better agreement with experiment.

The concentration of the radionuclide ^{54}Mn for the reactor outflow site was found to be practically constant with time and compares favourably with the theoretical prediction. In addition to ^{58}Co and ^{54}Mn small amounts of ^{60}Co were observed, while the amount of naturally occurring radionuclide ^{40}K varied in time with the salinity at each site.

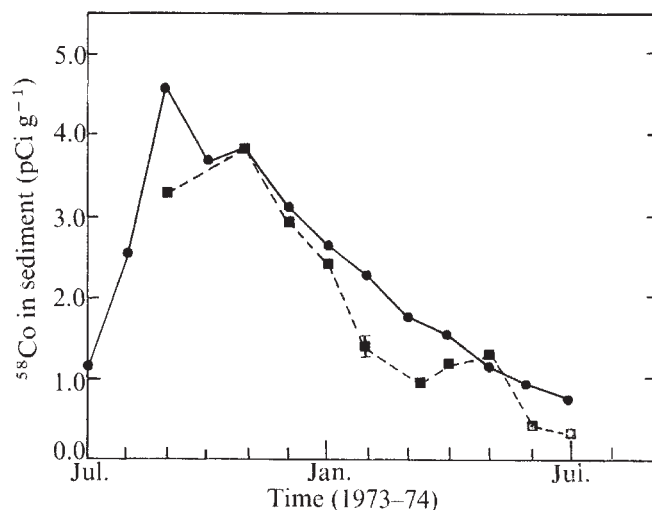


Fig. 3 ■, Measured ^{58}Co radionuclide concentration in sediment at the reactor outflow site. A typical 1σ error bar is shown. ●, Theoretical curve for ^{58}Co scaled by factor $U = 31 \times 10^{-12} \text{ g}^{-1}$ against time in months for the years 1973-1974.

The concentration of the radionuclide ^{58}Co in sediments is shown in Fig. 3. The broken lines represent the experimental values for this radionuclide and the solid line represents the theory. Again the decrease of ^{58}Co is more rapid than theory predicts, and in this case represents loss of radioactivity from the sediments by primarily physicochemical transport. The other three estuary sites had a factor of 10 less radionuclide than this particular site, but showed very similar variations with time.

Experimental measurements of sediments continued monthly after July 1974. These results are not, however, comparable with theory after this time because extensive construction in the estuary has temporarily changed and complicated the sedimentation.

For the sediments site at the outflow we observe the natural radionuclides ^{40}K and ^{210}Pb having average concentrations 5.7 pCi g^{-1} and 0.4 pCi g^{-1} respectively. We also observe the man-made radionuclides ^{58}Co , ^{60}Co , ^{54}Mn , ^{137}Cs , ^{134}Cs and ^{95}Nb with average concentrations of 4.9, 0.8, 0.2, 0.2, 0.4 and 0.8 pCi g^{-1} , respectively. These concentrations are presented to give a comparison of the natural and man-made radionuclides observed.

Since the theoretical and observed time dependence factors

seem to agree, it is possible to divide the sample concentration (pCi g^{-1}) at each site by the release of radionuclides from the reactor (Ci) to form a measured uptake ratio U . The U values for ^{58}Co in oysters at the different sites in order of increasing distance from the outflow were 7.6, 5.6, 2.6 and 1.3, where the units of U are $\text{g}^{-1} \times 10^{-12}$. These U values indicate most of the ^{58}Co is concentrated in the region of the outflow site decreasing rapidly with distance from the reactor. The U values of the other isotopes (^{60}Co and ^{54}Mn) show the same trend, both in the oysters and sediments. A first principles calculation of U values, which has not been done, must take into consideration the reactor radionuclide effluent inventory, the concentration factors for the uptake medium, and the physical oceanography⁷ of the estuary.

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Disordered drinking after abdominal vagotomy in rats

WHILE investigating the effect of abdominal vagotomy on satiety elicited by cholecystokinin¹, we noted that many vagotomised rats seemed to be undernourished and failed to regain their preoperative weight. Because of their low water-food ratio (vagotomised $1.3 \pm 0.5 \text{ ml g}^{-1}$, sham $1.8 \pm 0.1 \text{ ml g}^{-1}$, $P < 0.001$), we analysed their drinking behaviour in various conditions. We report here that abdominal vagotomy produces severe deficits in drinking behaviour that can be characterised as motivational.

Bilateral vagotomy was performed along the lower oesophagus in nine male rats (430-560 g) under Equithesin (Jensen-Salsbery Laboratories) anaesthesia. Sham vagotomy (manipulation of the oesophagus without nerve transection) was carried out in ten rats. Vagotomy was verified anatomically at the conclusion of the drinking tests without knowledge of the behavioural results. All nine vagotomised rats were completely disconnected.

Rats were housed individually, maintained on a 12-h light-dark cycle with continuous access to Purina pellets and tapwater except during drinking tests, when food was removed. Food and water intakes were measured daily for 1 week before and for 2 months after surgery. Drinking tests began 1 month after surgery when daily food and water intakes had stabilised.

Vagotomised rats drank much less water than sham rats during 24 h when no food was available (vagotomised $1.8 \pm 0.4 \text{ ml}$ per 100 g body weight (BW), sham $6.1 \pm 0.8 \text{ ml}$ per 100 g BW, $P < 0.001$; control data, Fig. 1). After 24 h water deprivation (with food available), vagotomised rats drank as much in 2 h as sham rats (vagotomised $2.4 \pm 0.3 \text{ ml}$ per 100 g BW, sham $2.9 \pm 0.1 \text{ ml}$ per 100 g BW) although five of the nine drank less than the smallest intake of sham rats. Vagotomised rats did not drink normally (Fig. 1) in the 2 h after cellular dehydration was produced by hypertonic saline

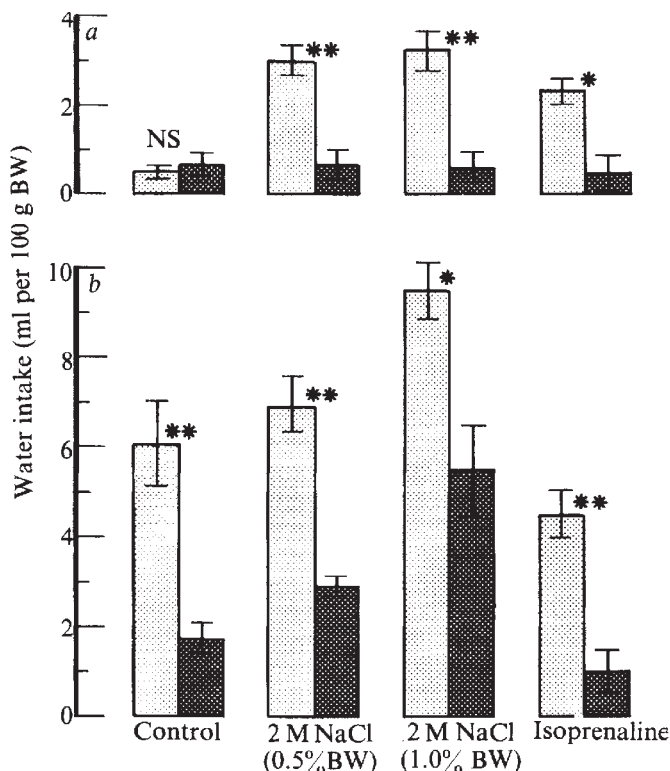


Fig. 1 Water intake ($\pm$ s.e.) for sham (light bars) and vagotomised (dark bars) rats in 2 h (a) and 24 h (b) after NaCl or isoprenaline (0.16 mg per kg BW). There were at least eight rats in each group in control and NaCl conditions. There were four vagotomised and five sham-operated rats treated with isoprenaline. Variance is expressed as s.e. All statistical comparisons were made with a two-tailed *t*-test. NS, Not significantly different; * $P < 0.01$; ** $P < 0.001$.

(2 M NaCl, 0.5 or 1.0 ml per 100 g BW, intraperitoneally). The deficit was severe: two of the nine did not drink after 0.5% BW NaCl (0.5 ml per 100 g BW) and five did not drink after 1.0% BW NaCl (1.0 ml per 100 g BW). None of the lesioned rats seemed depressed by the saline load.

Four hours after salt loading, vagotomised rats still drank less (unpublished data), but by 24 h their intake above control was equal to that of sham rats (Fig. 1). These 24-h intakes should not be interpreted as drinking in response to intracellular dehydration alone, because urinary excretion of solute load probably also led to extracellular dehydration.

In response to isoprenaline², vagotomised rats also failed to drink normally: three did not drink and the other two drank less and had longer latencies than sham rats (Fig. 1).

Polyethylene glycol also elicits drinking through extracellular³ and possibly intracellular⁴ stimuli. Five vagotomised and four sham-operated rats were injected with polyethylene glycol (5 ml of 30% w/w solution in 0.9% NaCl, subcutaneously) and immediately offered water. After 8 h, three of five vagotomised rats had not drunk. By 24 h, however, all rats had drunk, but group intakes differed significantly (vagotomised 12 ± 2.6 ml, sham 22 ± 2.0 ml, $P < 0.02$). When NaCl (0.9%) was offered to drink 24 h after injection of polyethylene glycol, all rats drank large volumes in the 1-h test (vagotomised 38.4 ± 9.2 ml, sham 34.6 ± 5.7 ml). The normal intake of saline and the low intake of water after polyethylene glycol demonstrates that the effect of vagotomy on water intake cannot be due to painful or uncoordinated oesophageal movements.

In summary, bilateral abdominal vagotomy abolished the drinking response to acute cellular dehydration and to isoprenaline, decreased drinking in the absence of food and after polyethylene glycol, decreased the water-food ratio, and tended to decrease drinking after water deprivation. Transection of both vagal trunks seems to be necessary for these deficits,

because drinking was normal in 2 rats with a large vagal connection persisting between oesophagus and stomach (unpublished). The critical region of vagal innervation that must be removed to obtain the drinking deficits was not determined, but the level of section preserved the vagal innervation of the liver and portal bed, which should have permitted normal function of postulated hepatic osmoreceptors⁵.

Such experiments have not been reported before in rats, but the effect of vagotomy on thirst has been studied extensively in the dog. The results are different and seem to depend on the level of vagal section: abdominal vagotomy increased the size of a bout of drinking, but did not alter the 24-h water-food ratio⁶; thoracic vagotomy did not change the drinking response to hypertonic saline⁷, but cervical vagosympathectomy enhanced drinking to hypertonic saline⁸ and blocked the ability of extracellular dehydration (haemorrhage) to decrease the osmotic threshold for drinking to hypertonic saline⁸. This last effect is consistent with the decreased responses to the extracellular challenges of isoprenaline and polyethylene glycol reported here, but none of this work in the dog predicts the failure of drinking after hypertonic saline that we found.

It is not likely that these drinking deficits result from the removal of efferent vagal influence, because peripheral anticholinergic block produced by methylatropine or methylscopolamine does not diminish drinking after hypertonic saline^{9,10} or water deprivation¹¹. Furthermore, the peripheral and central anticholinergic effects of atropine do not block drinking after isoprenaline².

If peripheral anticholinergic block mimics all the effects of removal of vagal efferent function, then the failure of anticholinergics to reproduce the effects of abdominal vagotomy on drinking suggests that the critical lesion is the loss of vagal afferent stimuli. The role of vagal afferent stimuli from the abdominal viscera in mediating thirst is not known. Fitzsimons¹² has suggested the importance of vagal afferent stimuli, originating in the walls of the great vessels and atria, for eliciting thirst after extracellular dehydration. These regions, however, remained innervated in our vagotomised rats.

Although we do not know if vagotomy abolished pancreatic osmoreceptor¹³ or mesenteric vascular volumetric stimulation, the fact that vagotomy abolished or attenuated drinking to a variety of stimuli suggests a deficiency in the motivation for drinking. The clearest example of the decreased responsiveness which characterises this lack of motivation is the drinking behaviour of vagotomised rats during 24 h when food was not present: they drank one-third as much as sham rats (Fig. 1).

In these experiments, the motivational deficit seemed to be restricted to drinking, because the 24-h food intake of vagotomised rats in the absence of water was not different from that of sham rats (vagotomised 3.8 ± 0.3 g per 100 g BW, sham 3.7 ± 0.1 g per 100 g BW). These data are not conclusive, however, and the specificity of the motivational deficit requires more rigorous testing.

Ball reported previously a motivational defect after abdominal vagotomy¹⁴. He observed elevated thresholds for lateral hypothalamic stimulus-bound eating and self stimulation in vagotomised rats. Ball suggested that this decreased excitability of the lateral hypothalamus after abdominal vagotomy was evidence for the importance of a vago-vagal, positive feedback loop in the mediation of these stimulus-bound behaviour patterns. Our interpretation of the results with drinking behaviour is analogous to Ball's suggestion for feeding and self stimulation.

If the disordered drinking observed after vagotomy were the result of elevated lateral hypothalamic thresholds, lateral hypothalamic damage should produce a similar deficit in drinking. This is confirmed by Stricker¹⁵, who reported that rats recovered from lateral hypothalamic lesions did not drink after polyethylene glycol, ligation of the inferior vena cava or hypertonic saline during relatively brief (1–6 h) testing conditions, but did drink significant amounts of water by 24 h after treatment.

Although the drinking deficits after abdominal vagotomy and lateral hypothalamic lesions seem similar, it remains to be determined if this behavioural similarity signifies an important neurological relationship between afferent vagal stimuli from abdominal viscera and lateral hypothalamic integration of drinking behaviour.

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Fast body turns in a cichlid fish

THE African cichlid fish *Haplochromis burtoni*, is sexually dimorphic. The female is smaller and of an homogeneous beige colour, whereas the male has bright colour patterns which he can turn on and off quickly. These are important signals, particularly during aggressive interactions¹, since these fish rely extensively, although not exclusively, on visual cues in their social behaviour². Sound production does not seem to be involved³, but chemical stimuli are important in male–female encounters during reproduction⁴. Colony observations in aquaria have shown that after the disruption of an established social situation, there is considerable behavioural interaction until the new social order is stabilised. Subsequent behavioural interaction is much less frequent, although a rank order is maintained. Since most social encounters seem to depend on visual cues, it seemed likely that vision was involved in the subtle interactions which characterise behaviour in an established social order. I have therefore monitored and analysed the horizontal eye movements of male *H. burtoni* during social encounters. I found a new kind of very fast body movement, during which the eyes were held fixed, which are correlated with specific types of behavioural interactions.

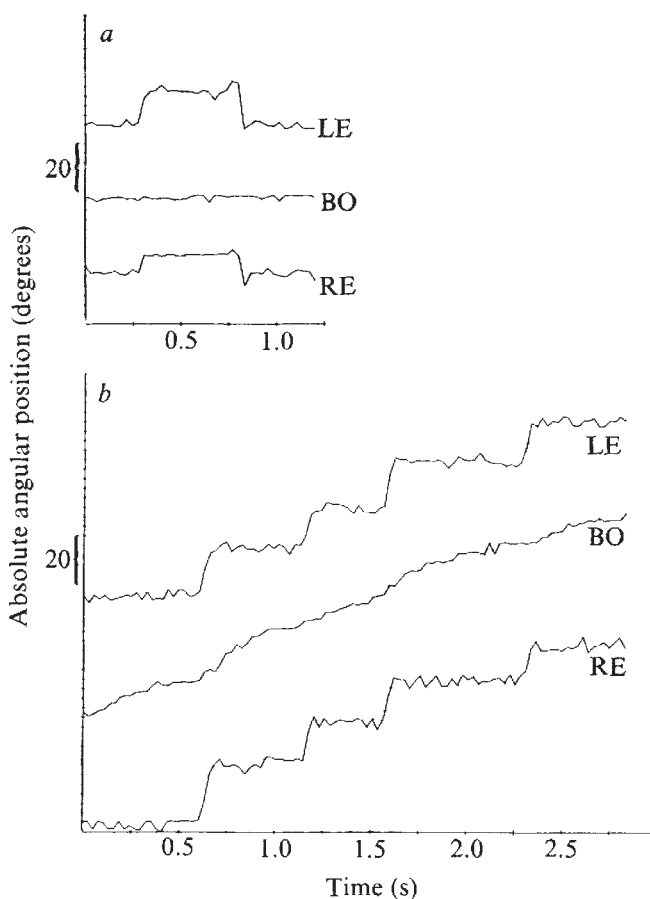
Mature male *H. burtoni* (7.0–7.5 cm long) were fitted with eye stalks by an adaptation of Easter's technique⁵. Eye stalks were made from black shrink tubing (0.5 mm diameter $\times$ 4.0 mm) by squeezing one end closed with heated tweezers. Chewing gently on the stalks for a few minutes softened them so that they could be attached by suction to the upper or rear edge of the fish's cornea. The obstruction to the visual field was 10% or less. The fish were placed in a cylindrical Plexiglas tank (50 cm diameter $\times$ 15 cm) with a transparent floor. The outer circumference was covered with opaque material, and the tank was illuminated from above through a translucent cover. Normally, three to five fish with eyestalks were filmed from below as reflected in a front-surfaced mirror, with a Super-8 Beaulieu camera at

60 frames s⁻¹. An electronic counter driven by a pulse generator at a known frequency was filmed simultaneously for identification of individual frames and calibration of filming speed.

The data were analysed by projecting the film frame by frame and viewing it through a clear Plexiglas disk with parallel lines etched on it and mounted on a potentiometer. The lines were aligned with the object of interest—an eye stalk or the body position—by rotating the disk. The body position was taken as an axis perpendicular to a line drawn between the two eyes. A voltage proportional to the angle being read was punched on paper tape, which was later fed into a computer (DEC 11/40) and the angular orientation plotted against frame (time). The orientation of the body and of each eye of the fish could thus be measured with respect to the tank for each frame. This angular measurement was repeatable with an accuracy of $\pm 1^\circ$.

Three types of motion could be distinguished: eye movements without associated body movements, coordinated eye and body movements (both of which have been described before), and fast body movements with no corresponding eye movements. Figure 1a illustrates the first type in which the body remains stationary while the eyes flick rapidly to new positions and, after some time (in this case about 0.5 s), return to approximately their original positions. A typical example of the coordinated eye–body movement is shown in Fig. 1b. A binocular saccade normally preceded a body turn, and the eyes rotated in the opposite direction relative to the body as turning continued, so that they remained in a nearly constant orientation in space. Both types of eye movement have been described for goldfish^{6,7}.

Fig. 1a. Absolute angular position of the left eye (LE), body (BO), and right eye (RE) as a function of time. The body remains stationary while the eyes rotate to a new position, returning after 0.5 s. b. Coordinated eye–body movement during a slow body turn. In this saccadic eye movement, the eye flicks to new fixation points in space which are maintained as the body turns.



The details of such eye movements in *Haplochromis* and experimental analysis of their control and use will be described elsewhere.

Two examples of the third type of motion are shown in Fig. 2. During these fast body turns, the eyes remain fixed relative to the body. In the first case there are two successive fast turns, both about 40° , separated by 0.2 s. Approximately 0.25 s after the second turn, the animal made a typical saccadic eye movement and corresponding slow body rotation. In the second example, the fish made one large fast turn (105°), followed in 0.1 s by a smaller turn (20°). Approximately 1 s later, the fish made a binocular saccade coordinated with a slow body turn. After the fast turn, if slow body movements occurred, the eyes seemed to be fixated in space (compare eye position in Fig. 2a between the two turns).

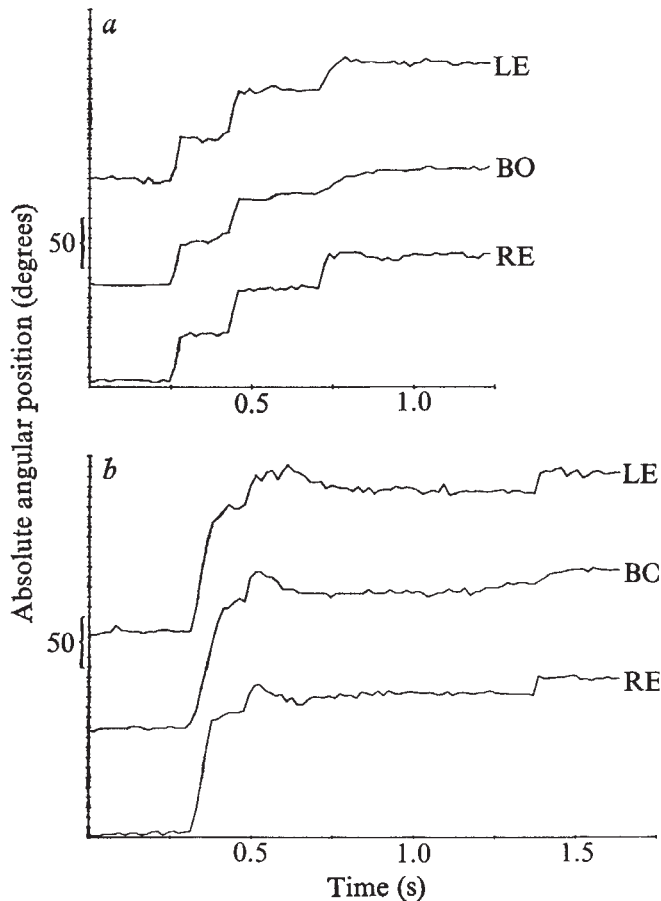


Fig. 2 *a*, Two successive fast body turns followed by a saccadic eye movement (at 0.75 s). During the fast turns there is no eye motion relative to the body. *b*, One large fast turn, followed by a small one and then later (at 1.3 s) a saccadic eye movement.

Both the angular size and angular speed of fast body movements far exceed those of body movements associated with saccadic eye motion (Fig. 3). In fact, the rotational velocity of the body during a fast turn approximates that of the eye during a typical saccadic flick (compare Fig. 2).

Compared with a normal aquarium, the tank I used had a relatively small volume for habitation by several fish, and this artificial crowding intensified the behavioural interaction in response to alteration of the social situation. After a fish had been introduced into the tank, there was a short quiescent period of adaptation to the new environment of about 10 min. Some time thereafter, vigorous fighting ensued (threat displays, biting, chasing, mouth-to-mouth biting and pulling), which seemed similar to territorial disputes in larger aquaria. The fighting continued (0.5–1.5 h) until one fish clearly dominated the tank, having won in

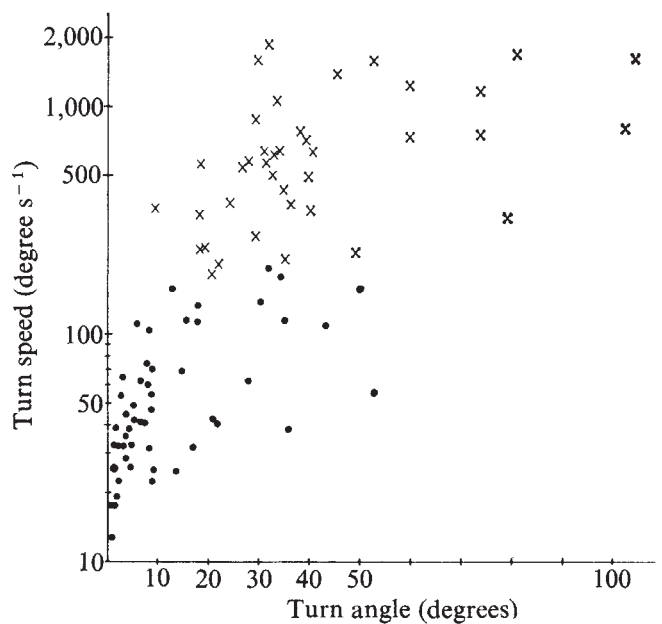


Fig. 3 For body movements, the turn size (degree) is plotted against the turn velocity (degree s^{-1}). Body movements for both fast turn ($\times$, $n = 39$) and saccadic eye movements ($\bullet$, $n = 52$) are shown.

paired encounters with all contenders. Only rarely was this social order then changed without the addition or removal of fish.

The fast turns were recorded only during these paired encounters. The animals oriented themselves for subsequent bites, threats and so on with a fast turn just before the behavioural act. The two fast turns in Fig. 2a, for example, preceded a vigorous bite into the opponent's tail, after which the attacked animal fled. If the dominant animal was removed from the tank, the remaining fish resumed fighting and performing the corresponding fast turns until a new male dominated.

It is not yet clear whether the fast turn serves as a behavioural signal, intentional or otherwise, in addition to its orienting function. These turns produce a unique sound (Fig. 1 of ref. 3) and can be recognised by a trained observer. The combined auditory and visual information could serve to alert fish nearby.

The fast turns are neither identically long nor large, but seem to be matched to the behavioural situation, which suggests that there is a large coordinated muscular discharge under rather precise control. Two factors make control of this turn unique: first, during the turn, the eyes are fixed with respect to the body, and second, the time spent turning is very short. Both imply that the fish may be making the turns under open loop control, without use of visual or other feedback, a possibility which must be tested experimentally.

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Multicomponent alarm pheromones of the weaver ant

THE exocrine glands of ants are often found to contain blends of volatile chemicals¹, but only rarely have distinct releaser properties been ascribed to different components of a single gland²⁻⁴. The African weaver ant *Oecophylla longinoda* is notoriously aggressive in defending its territory, and communication of alarm is rapid and efficient. Colony defence is carried out almost exclusively by the major workers, the minor caste remaining within the leaf-nest until this is directly disturbed⁵. This marked difference in behaviour may reflect a biochemical polymorphism, although this has not yet been reported for alarm pheromones of ants⁶. We have therefore initiated a detailed study of exocrine secretions that could be implicated in the control of alarm and defence in this ant. In the mandibular gland we have found over thirty compounds, some of which control separate aspects of behaviour. The mandibular gland contents also differ markedly among castes.

Excited major workers often give out a fragrant but musty odour, which originates from the mandibular glands. Crushed heads or mandibular gland reservoirs release a sequence of behaviour, which may be broken down into four components: alerting, in which the ants raise their heads and open their jaws, and may snap at nest-mates nearby, attraction towards the source of the chemicals, arresting of locomotion at close range, and biting. The last is a particularly marked reaction, in which the ant often pulls for several minutes if resistance is offered by the object being bitten. Prey is normally immobilised by a number of ants pulling from different directions. Gas chromatography of single mandibular glands indicated that at least 33 chemicals are present at or above a level of 5 ng per gland (Fig. 1). The major components were identified as hexanal (1) and 1-hexanol (7). Pure samples of these were presented to laboratory cultures of the ants in capillary tubes, which give a release rate comparable with that produced by the ants⁷. Hexanal releases alerting and alarm with little evidence of directional orientation, while a source of 1-hexanol attracts ants towards it from a radius of about 10 cm, but the ants are alarmed and repelled at a distance of a few mm from the source. The biting behaviour is apparently under the control of less volatile components, since this can be observed in response to the mandibular gland secretion after

it has been allowed to evaporate for a few minutes, and no longer releases alarm.

Small quantities of the components eluting after 1-hexanol were collected as broad fractions by solid-sample preparative gas chromatography⁸ and were presented to foraging major workers. The fraction containing peaks 18-23 was most active in releasing biting (Wilcoxon matched-pairs signed-ranks test, $T=1$, $N=8$, $P<0.01$, one-tailed test) compared with controls. This fraction was further divided into individual components, and peaks 18 ($T=10$, $N=10$, $P<0.05$) and 23 ($T=0$, $N=7$, $P<0.01$) were most active. They were shown to be 3-undecanone (18) and 2-butyl-2-octenal (23) by mass spectrometry and comparison with commercial (18) and synthetic (23) samples (full details of the structure elucidation will be published elsewhere). The combination of 1-hexanol, hexanal and 2-butyl-2-octenal has also been found in the coreid bug *Amblypelta nitida*⁹; a linked biosynthesis seems likely in both species. 2-Butyl-2-octenal and 3-undecanone were tested in the quantities found in a single gland (50 ng and 20 ng respectively). Both released biting, although the aldehyde was more active than the ketone ($T=14$, $N=12$, $P<0.05$, two-tailed test). The ketone is also an attractant over a 2-cm range.

The behaviour released by the total mandibular gland secretion has been compared with that described for the four chemical stimuli we have isolated (Fig. 2). It seems that a sequential message is produced from the active spaces¹⁰ of these chemicals determined both by their individual volatilities within the mixture and their threshold concentration for the particular response. In still air the spaces will be concentric about the point of deposition of the secretion detailed in Fig. 2. Behavioural tests indicate that hexanal has the most rapidly expanding activity; this is followed by 1-hexanol, which attracts the ants towards the smallest active

Fig. 1 Gas chromatography of the head of a single major worker of *Oecophylla longinoda*; 1.5 m by 6 mm outside diameter glass column packed with 10% polypropylene glycol adipate, the oven held at 80 °C for 5 min, then programmed to 168 °C at 4 °C min⁻¹, injection (arrowed) by solid-sample¹¹. Only those components discussed in the text are numbered.

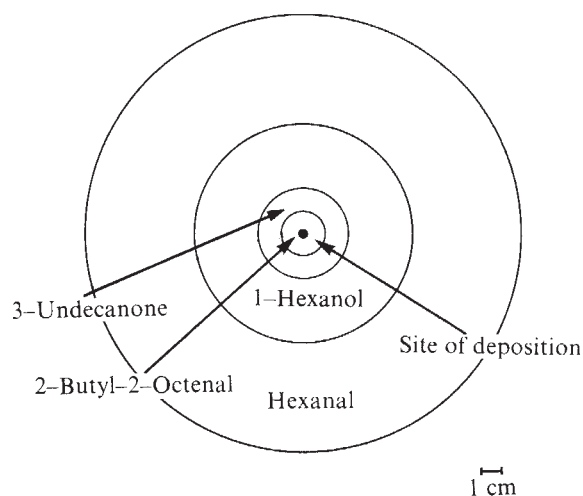
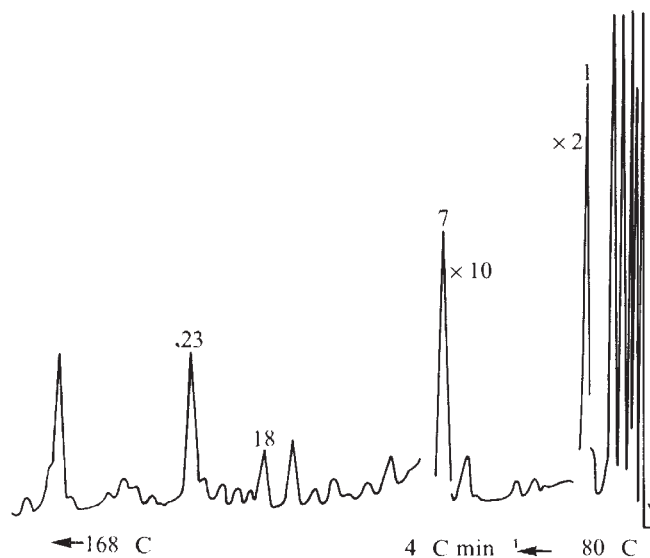


Fig. 2 Active spaces of four mandibular gland components 20 s after deposition. (1) Hexanal and 1-hexanol were presented to foraging workers as pure compounds in glass capillary tubes (1 mm inside diameters) open at one end, which give a release rate comparable with that produced by the ants⁷. After 20 s, the alerting and alarming activity of hexanal extended to a radius of 10 cm, and orientation and running towards a source of 1-hexanol began at a radius of 5 cm. (2) 3-Undecanone and 2-butyl-2-octenal were presented on small flags of filter paper which were first washed in redistilled methylene chloride and were fixed to the foraging table on fine entomological pins. Test solutions of 20 ng of 3-undecanone and 50 ng of 2-butyl-2-octenal (the quantities found in a single mandibular gland of a major worker from the colony under test) were measured on to the filter paper and the solvent (hexane) allowed to evaporate. Control filter papers were treated with solvent only. Over a 20-s period, ants were attracted to 3-undecanone over a 2-cm radius, but biting behaviour in response to either 3-undecanone or 2-butyl-2-octenal was observed only within 1 cm of the pin. Observations on behaviour in response to the total mandibular gland secretion showed that the active spaces of the components were approximately the same in combination as they were when tested individually.

spaces, those of the biting markers. 3-Undecanone may provide an additional directional cue at closer range. The effects of the individual components seem to be additive, rather than synergistic, as observed in two laboratory cultures, collected from the forest areas of Ile-Ife and Ibadan, Nigeria. Pure 3-undecanone released biting in a third culture, collected about 700 km to the north, in savanna at Mokwa, but 2-butyl-2-octenal was inactive alone. A sample containing an equal amount of the aldehyde and 1-hexanol restored the activity to the same level as the ketone; mandibular glands of major workers collected from Mokwa differ in containing 1-octanol and 1-nonanol in addition to 1-hexanol, and these less volatile alcohols may be more persistent synergists. In either case a rapid communication of alarm is achieved, while the attack response is localised to the object on to which the mandibular gland secretion has been deposited.

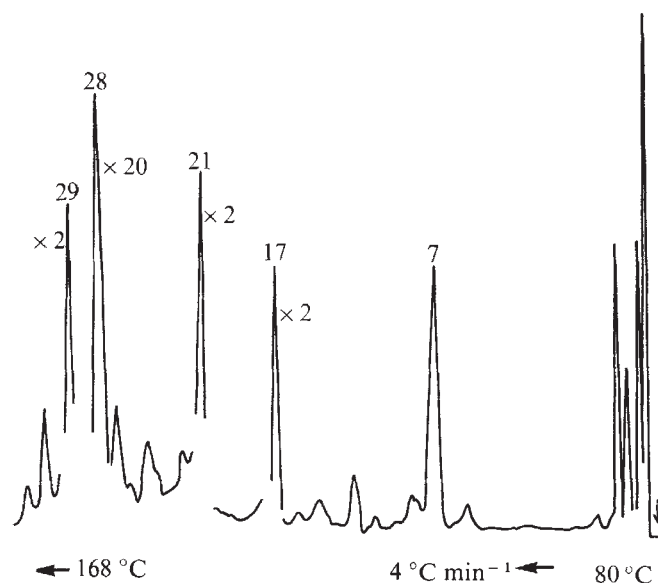


Fig. 3 Gas chromatography of the head of a single minor worker head of *Oecophylla longinoda*; experimental details as for Fig. 1, vertical scale increased $\times 2$.

Gas chromatography of single heads of minor workers (Fig. 3) indicates considerable differences from majors; the main component (28) was identified as nerol, with geraniol (29), 1-hexanol (7), 1-octanol (17) and 1-nonanol (21) also present. Hexanal, 3-undecanone and 2-butyl-2-octenal seem to be absent at the level detected (~ 2 ng per ant). Minor workers exposed to either 1-hexanol and nerol in capillary tubes are repelled, and thus displaced from sources of disturbance. Major workers are attracted and arrested at about 1 cm from a source of nerol in a capillary, but this reaction is not observed until about 2 min after the tube is placed on the foraging table, which indicates a high threshold concentration. A disturbance within the confined space of the leaf-nest would result in a rapid and rather uniform increase in the concentration of 1-hexanol, decreasing its effectiveness as an orientation stimulus. For the same release rate, the active space of nerol is smaller and slower to expand, and this could enable excited minor workers to be located by majors.

Two further methods of alarm communication are found in this species. Major workers strike their bodies against the leaf-nest when it is disturbed, the sound being likened by Weber⁵ to dry peas dropping on to a plate, and several major workers then appear at the nest entrance. The abdominal defensive secretion also releases alarm behaviour, but only two compounds seem to be involved. A mixture of formic acid, found in the poison gland, and *n*-undecane from the Dufour gland, releases alarm, attraction and attack. The mixture is considerably more effective than either component tested separately. The

poison and Dufour gland secretions of major and minor workers are qualitatively similar.

This study has focused on only a few of the many chemicals which may have a role in the control of alarm and defence behaviour in this species, and suggests that the simple concept of an alarm pheromone governing behaviour by virtue of one major component may be inappropriate for ants. It also demonstrates that biochemical polymorphism and population differences exist and should be taken into account in future studies.

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Penetration of mid-gut cells of *Glossina morsitans morsitans* by *Trypanosoma brucei rhodesiense*

UNTIL recently, the developmental cycle of African sleeping sickness trypanosomes in tsetse flies (*Glossina* spp.) was thought to follow exclusively the pattern described by Robertson¹. She suggested that trypanosomes ingested by a fly taking an infected blood meal multiplied in the gut and eventually invaded the salivary glands directly without leaving the confines of the alimentary tract. Inside the salivary glands the trypanosomes could develop into metacyclic forms capable of initiating fresh infections in mammals. In 1972, Mshelbwala² described stages of *Trypanosoma brucei* he had found in the haemolymph of three species of tsetse fly, and suggested that a more direct route from the gut to the salivary glands existed. According to his hypothesis, the trypanosomes from the gut of the fly penetrated the gut wall and entered the haemocoel; they then migrated to the salivary glands directly through the haemocoel, penetrating the glands and so completing the cycle of development in the fly. At this time there was no direct evidence that salivarian trypanosomes could penetrate the gut wall of their insect vector. In this communication we present electron microscopic evidence that *T. b. rhodesiense* penetrates the mid-gut cells of *G. morsitans morsitans*.

Tsetse flies are difficult to infect with trypanosomes of the *T. brucei* complex, and one suggested³ reason for this is failure by the trypanosomes to complete the biochemical and morphological changes by which the bloodstream trypanosomes transform to mid-gut forms. To overcome this difficulty, we cultured *T. b. rhodesiense* *in vitro*, producing forms which are believed to correspond to mid-gut forms, which were then fed to the flies.

Laboratory-fed *G. m. morsitans* were fed artificially through agar-Parafilm membranes⁴ on a diet of defibrinated horse blood containing approximately 2×10^5 culture-form trypanosomes per ml. These culture forms were grown to mid-log phase in a human blood lysate broth medium⁵, and were derived from a strain of *T. b. rhodesiense* (180 TSI) isolated by Dr W. E. Ormerod from a human patient in Botswana. The flies which provided material for the electron micrographs presented here were fed on six occasions. The first three feeds

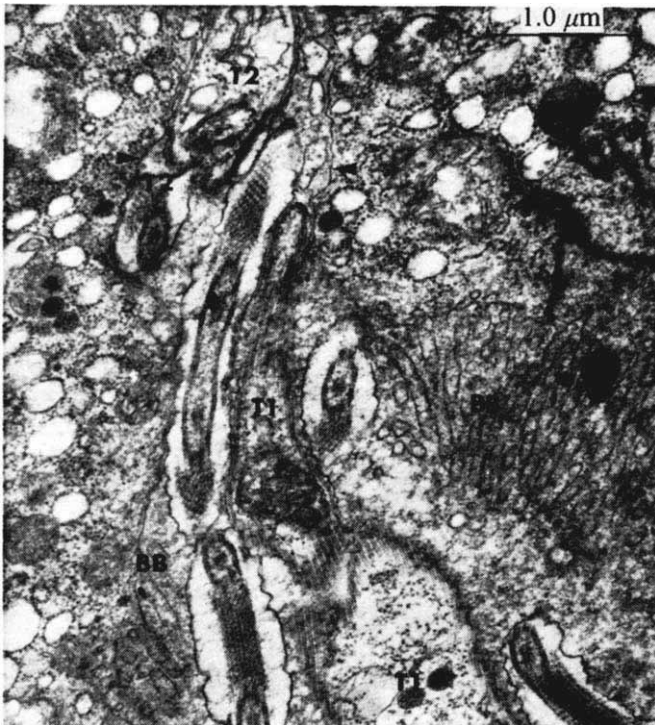


Fig. 1 Two trypanosomes (T1 and T2) invaginating the brush border membrane (BB). The walls of the resulting pocket are arrowed.

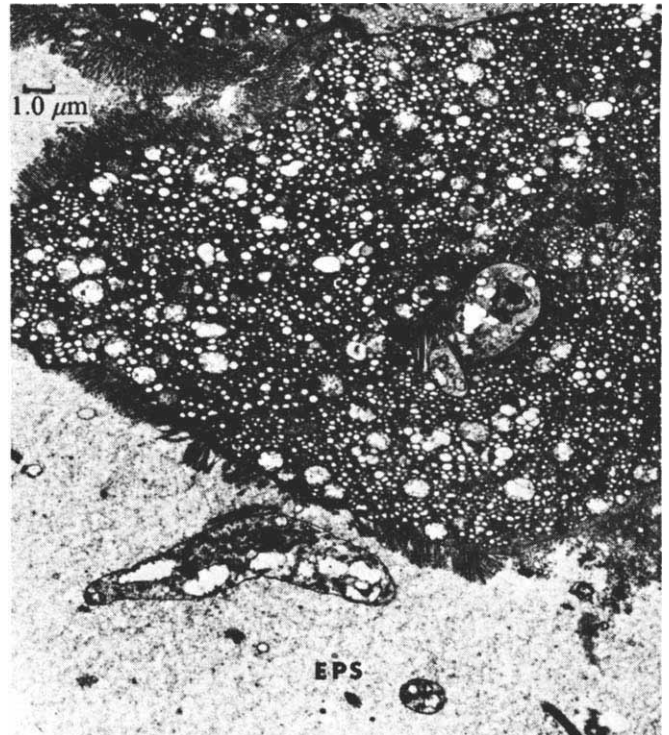


Fig. 3 Two trypanosomes within the cytoplasm of a mid-gut cell. Other trypanosomes are visible in the ecto-peritrophic space (EPS).

Fig. 2 Transverse section of two invaginated pockets in a gut-cell containing numerous trypanosomes. The membranes of these pockets are clearly seen unlined with microvilli.

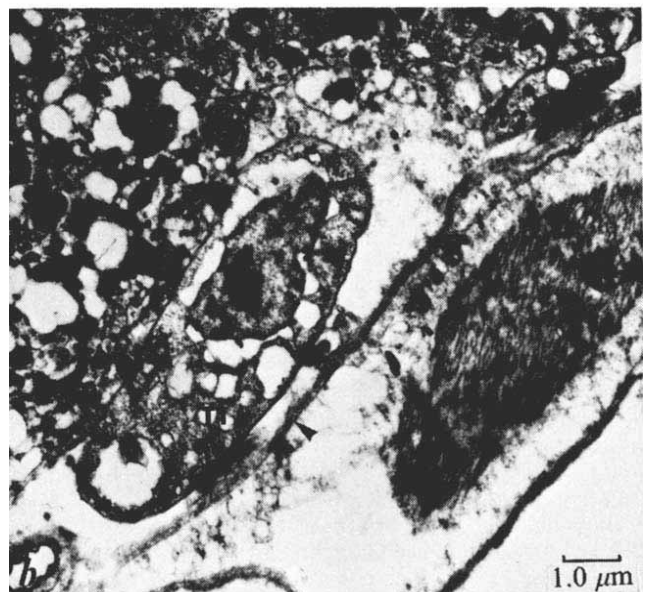
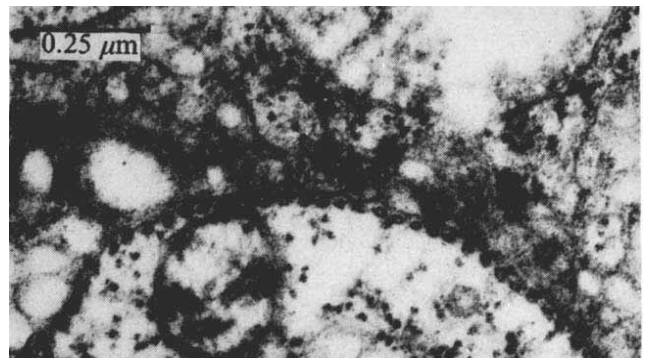
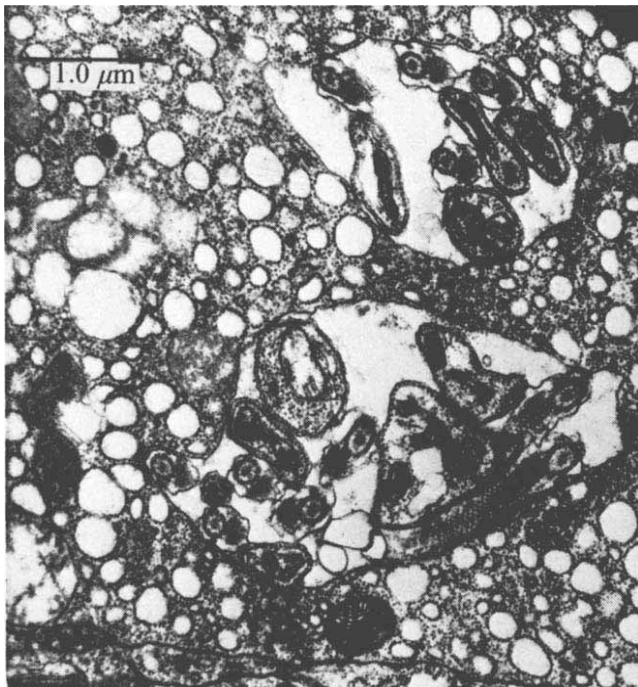


Fig. 4 *a*, Higher magnification of a trypanosome pellicle (arrowed) showing the parasite within the gut cell and free of any host-cell membrane. *b*, Two trypanosomes lying between the mid-gut basement membrane and the membrane of the haemocoel (arrowed). All sections were stained with lead citrate.

were of trypanosome-infected blood, and the remaining feeds contained defibrinated blood alone. Twenty-one days after their first infected feed, the flies were killed and their guts dissected out. The guts were fixed in 3% (v/v) cacodylate-buffered glutaraldehyde (6 h at 4 °C), washed overnight in cacodylate buffer, postfixed in cacodylate-buffered 1% (w/v) osmium tetroxide and prepared for embedding in Araldite.

The electron micrographs presented here show trypanosomes in the ecto-peritrophic space of the mid-gut, close to the brush border of the gut epithelium, and penetrating, flagellum first, the brush border membrane. This membrane, now surrounding the trypanosome flagellum (Fig. 1), has the appearance, when viewed in transverse section (Fig. 2), of a vacuole within the host cell containing numerous parasites. Once wholly inside the gut cell (Fig. 3), the trypanosomes lie free, no longer surrounded by host cell membrane (Fig. 4a).

Finally, the trypanosomes reach the basement membrane of the gut cell, which they penetrate, and come to lie in the space between the gut cell basement membrane and the haemocoel membrane which sheaths the gut (Fig. 4b).

It seems that entry of the trypanosomes into tsetse fly mid-gut cells is an active process on the part of the trypanosomes, and not a result of phagocytic action of the mid-gut cells, nor was evidence found of passive enclosure of trypanosomes between gut cells during the extension and contraction following blood meals. This view is supported by our finding that the intracellular parasites are in no way associated with the membranous system of the host cell; the electron microscopic appearance of the intracellular trypanosomes, and the discovery of undamaged parasites in the space between the gut cell basement membrane and the haemocoel membrane, suggest that the trypanosomes are not affected by the gut cells.

A route from the gut of an insect vector through the haemocoel to the salivary glands has been described for other parasitic protozoa. *T. (Herpetosoma) rangeli* migrates to the salivary glands of its reduviid bug vector in this manner, as do malaria sporozoites in the mosquito. Whether this route is important for sleeping sickness trypanosomes is unknown, but this is being investigated.

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T cells mediate transplantation tolerance

SEVERAL cellular mechanisms have been postulated to account for the phenomenon of transplantation tolerance in animals injected at birth with allogeneic or semi-allogeneic cells. These include deletion of the clone of specific alloantigen sensitive cells¹, continuous production of conventional blocking alloantibody² or anti-receptor antibody³ and the action of suppressor cells⁴. Although these phenomena have been demonstrated in tolerant animals, it has never been shown that either alone or in combination they are actually capable of inducing true transplantation tolerance. In the course of experiments designed to prove formally that recirculating small lymphocytes are responsible for first-set rejection of skin grafts, we obtained results suggesting that rapidly

recirculating cells from tolerant donors might be capable of actively transferring a state of transplantation tolerance (TT) to irradiated recipients^{5,6}. These results, which suggested that the transfer of TT was due to humoral or cellular suppressor mechanisms, were not consistent with the clonal deletion theory of tolerance. The present experiments were designed to confirm that TT is not merely the absence of reactivity to a given set of allo-antigenic determinants but an active suppression of reactivity to those determinants, and to determine whether T or B cells mediate this suppression.

Thoracic duct (TD) lymph contains both T and B cells and members of both these subpopulations continuously recirculate from blood to lymph⁷. Cells mediating allo-immune responses *in vivo* are among the populations which recirculate^{5,6}. The lymphoid tissues of rats whose own lymphocyte pools have been virtually eliminated by supra-lethal (1,000 rad) doses of radiation are still capable of supporting the recirculation from blood to lymph of injected TD lymphocytes. Either syngeneic or allogeneic TD lymphocytes injected intravenously into such rats can be recovered from a thoracic duct fistula uncontaminated by significant numbers of radioresistant host lymphocytes⁶. Because the modal transit time of T lymphocytes through lymph nodes is considerably shorter than that of B cells^{8,9}, peak recovery of injected T cells occurs before significant numbers of B cells begin to emerge (Fig. 1). Collections of lymph made for the first 24 h after injection yield approximately 20% of the injected T cells and virtually none of the injected B cells. We have examined the capacity of both unfractionated TD lymphocytes from tolerant donors, and pure T-cell populations separated from the former by this method, to mediate TT.

Thoracic duct lymph from 10 DA rats tolerant of PVG alloantigens was collected for the first 24 h after cannulation and the cells were washed twice. Aliquots of 3×10^7 cells were injected intravenously into sublethally (750 rad) irradiated DA rats freshly grafted with PVG and Lew skin. The remainder of the cells (1.25×10^8 per recipient) were injected intravenously into supra-lethally irradiated DA rats bearing thoracic duct fistulae, and collections of lymph

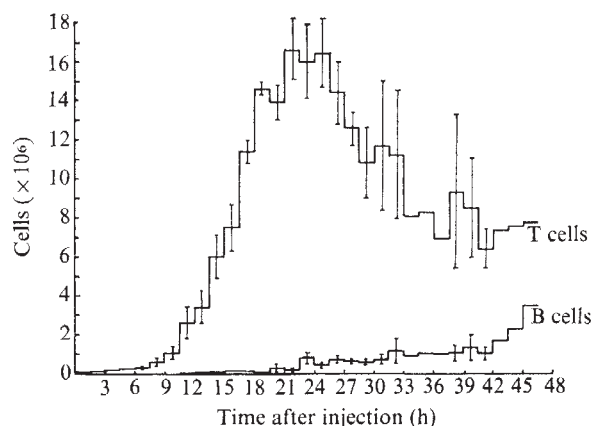


Fig. 1 Output of T and B cells in 1.5-h collections of TD lymph from supra-lethally irradiated rats (1,000 rad ⁶⁰Co γ radiation) injected intravenously with 1.25×10^8 syngeneic TD lymphocytes of which 44–48% were Ig positive. All rats were splenectomised at the time of cannulation to prevent temporary sequestration of injected TD lymphocytes in the splenic pool. Cells were counted in each collection and the proportion of Ig-bearing lymphocytes was determined by direct fluorescence using FITC conjugated rabbit anti-rat Ig serum diluted 1:30 (Burroughs Wellcome, lot No. K7284). T cells were positively identified in representative collections by indirect immunofluorescence using a 1:4 dilution of a rat anti-T-cell serum¹⁰ followed by two washes through foetal calf serum gradients and staining with the same rabbit anti-rat Ig serum as before. Error bars represent the standard errors of the arithmetic means of determinations on eight rats in four separate experiments.

made from these rats for the subsequent 0–22 h and 22–46 h. Cells in each of these collections were pooled and assayed for the proportion of T and B cells. Aliquots of 3×10^7 of these cells were injected intravenously into sublethally irradiated DA rats also bearing both PVG and Lew skin allografts (Fig. 2).

In order to determine the rate of spontaneous recovery of immune responsiveness in rats given 750 rad, groups of irradiated, unrestored rats were doubly grafted in the same way as the experimental groups. In addition, the competence of separated T cells from normal donors to mediate alloimmune responses was confirmed by restoring a further group of irradiated, doubly grafted recipients with 3×10^7 cells from supralethally irradiated DA rats given 1.25×10^9 TD lymphocytes from normal donors. The survival of skin grafts on rats given normal or tolerant T-cell inocula and on rats given no restorative inoculum are presented in Fig. 2. As the behaviour of grafts on rats given unfractionated TD lymphocytes, either normal or tolerant, did not differ from the results obtained with the respective purified T cells, the results of the former are omitted from the life tables for the sake of clarity.

In the unrestored sublethally irradiated control rats, spontaneous recovery of the immune response ensured that both grafts were eventually rejected, although the first-set allograft response was temporarily ablated. Median survival time (MST) for Lew grafts was 17.5 d; the MST for PVG grafts was 25.3 d (Fig. 2, *D* and *E*). When sublethally irradiated recipients were restored with cells from normal donors, whether unfractionated TD lymphocytes containing 40–50% B cells or purified T cells containing less than 0.3% B cells, the rejection times of both grafts were restored to normal (MST Lew, 8.5 d; MST PVG, 9 d) (Fig. 2, *A* and *B*). Previously reported experiments have established that the restored responses in sublethally irradiated grafted rats

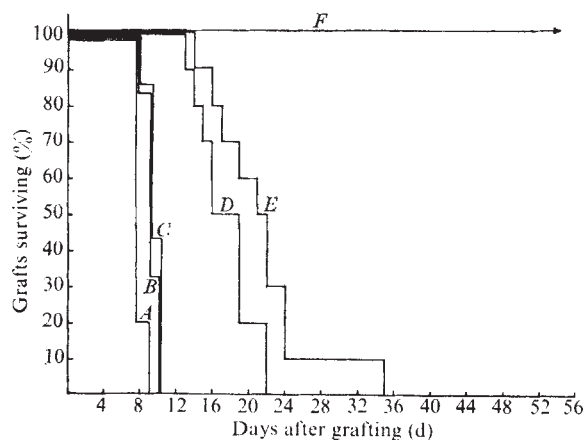


Fig. 2 Life table of graft survival in DA (Ag-B4) rats grafted with PVG (Ag-B5) skin on one flank and Lewis (Ag-B1) skin on the other within 20 h of sublethal irradiation (750 rad). *D* and *E*, rejection times of Lew and PVG grafts respectively on 10 rats. Rejection of both grafts is delayed by irradiation. *A* and *B*, rejection times of Lew and PVG grafts respectively on 10 rats given 3×10^7 purified T cells from normal donors (99.8% T cells). Rejection times of both grafts are restored to within the normal first-set range. *C* and *F*, rejection times of Lew and PVG grafts respectively on 11 rats given 2×10^7 purified T cells from tolerant donors. Six recipients were given the 0–22 h collection (99.7% T cells) and five rats were given the 22–48 h collection (96.1% T cells). The rejection time of Lew skin grafts was restored to within the normal first-set range in both groups. The rejection of PVG skin grafts in both groups was completely suppressed. These grafts are in impeccable condition at the time of writing (> 100 d). Tolerant donors had borne perfect PVG skin grafts for at least 50 d. Pooled TD lymphocytes from these donors were assayed for graft *versus* host activity in (DA $\times$ PVG) F_1 and (DA $\times$ Lew) F_1 hybrids using the popliteal lymph node assay¹⁶. Absence of responses in (DA $\times$ PVG) F_1 and large responses in (DA $\times$ Lew) F_1 confirmed true tolerance in the donors.

given TD lymphocytes are mediated by the restorative inoculum^{3,6}, and that if the clone of cells reactive to PVG antigens is deleted from such a restorative inoculum by filtration through supralethally irradiated PVG rats, the restoration of first-set rejection of PVG skin grafts is specifically abolished; these grafts are rejected with the same MST as in unrestored sublethally irradiated animals⁶.

The effect of inocula of cells tolerant to PVG antigen in sublethally irradiated recipients was markedly different (Fig. 2). The recovery of reactivity to PVG antigens was not merely delayed, as in unrestored recipients or recipients of clonally deleted cells, but the PVG grafts survived indefinitely in impeccable condition. This phenomenon is not explicable in terms of absence of the clone of allo-antigen sensitive cells but implies that populations of tolerant cells contain cells capable of active, specific suppression of the regenerating immune response in animals recovering from radiation.

Both suppressor T cells and specific B-cell products have been implicated as mediators of tolerance in a variety of systems¹⁰. Tolerant TD lymph contained the same proportion of B cells, 40–50%, as does normal TD lymph. When passaged from blood to lymph in supralethally irradiated rats, 99.7% of the tolerant TD lymphocytes collected within 24 h of injection were T cells. Both this population and later collections (96.1% T cells) were just as efficient as unfractionated, tolerant TD lymphocytes in adoptively transferring specific tolerance (Fig. 2, *C* and *F*). The original donors of tolerant TD lymphocytes had been given 5×10^7 (DA $\times$ PVG) F_1 bone marrow cells by intra-cardiac injection within 24 h of birth and grafted with PVG skin at 12 weeks. The extent of chimaerism in these donors (assessed by dye-exclusion cytotoxicity assays using specific cytotoxic alloantisera of high titre) was below the detection threshold of 5%. This is in agreement with the low levels of chimaerism in tolerant mice reported by other workers using different assay systems^{11,12}. Experiments were nevertheless done to exclude the possibility that chimaeric cells in the restorative inocula from tolerant donors could have been responsible for the induction of tolerance in irradiated hosts. The rejection of PVG skin grafts by sublethally irradiated rats was completely unaffected by giving restorative inocula of either 3×10^5 or 3×10^6 (DA $\times$ PVG) F_1 cells. The latter would be equivalent to 10% chimaerism. This makes it unlikely that the transfer of chimaeric cells is responsible for the suppressor activity of purified T-cell populations from tolerant donors.

A dose of 750 rad is far in excess of that necessary to abolish completely alloantibody synthesis in rats¹³, and restorative inocula containing 20–40 times as many B cells as were present in the restorative inocula used in these experiments will not restore antibody synthesis to such animals¹⁴. It therefore seems clear that the injected syngeneic T cells alone must have been responsible for the specific suppression which induced tolerance in the irradiated recipients of tolerant T-cell inocula. This result implies that true transplantation tolerance is a positive immune response mediated by T cells which belong to the recirculating pool of small lymphocytes. The origins, life span and mode of action of these cells will obviously be the subjects of further study.

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Clonal growth of Hodgkin cells

ALTHOUGH several cell types derived from biopsies of patients with Hodgkin's disease have been grown *in vitro*^{1,2}, there has been no convincing experimental evidence for the identity and origin of such cells. We wish to present data demonstrating clonal and continuous proliferation of Hodgkin cells, their relationship to Reed–Sternberg cells and their probable origin from B lymphocytes.

Cells were obtained from pleural effusions of two patients with terminal Hodgkin's disease. The lymph node histology at autopsy of both cases was lymphocytic depletion. The cases were selected on the basis of an abundance of Hodgkin and Reed–Sternberg cells in the pleural fluid (Fig. 1): the large Reed–Sternberg cells were binucleate or multinucleate, the nuclei having a coarse nuclear chromatin network and large blue-stained nucleoli. Their cytoplasm was pale and often vacuolated. Cells considered to be Hodgkin cells had the same nuclear and cytoplasmic characteristics, but were mononuclear.

Cells were separated from the pleural effusions by the Ficoll gradient method and cultured in diffusion chambers as described for human bone marrow and peripheral blood cells^{3,4}. Figure 2 shows similar growth curves of the cells

from both cases during 11 d of culture. An initial decrease in total cell numbers, levelling off on days 2–5, was followed by a steady exponential increase. Net gain of total cells was observed after 8 d in culture in case 2 but not in case 1. During exponential growth, cell doubling times were 48 and 72 h respectively in the two cultures.

After 11 d of culture the cell contents of single diffusion chambers from case 2 were transferred into five to eight new chambers. Subculture was repeated at 11–15 d intervals. This cultured cell line has now been maintained for 4 months with continuous production of cells. Throughout the culture period the proliferative properties of the total population, the morphology of the Hodgkin and Reed–Sternberg cells (Fig. 1) and their proportional distribution (~30:1) remained unchanged.

In both cases the EBNA-test⁵ for detection of the Epstein–Barr virus (EBV) genome was negative on the fresh pleural effusion cells.

For chromosome analyses of fresh pleural cells and of cells cultured in diffusion chambers, the direct method was used as before⁶. Contents of three to five diffusion chambers were pooled. G banding was obtained by the trypsin–saline–Giemsa technique⁷. The number of metaphases analysed and the numerical chromosomal data are shown in Table 1. The karyotype of the predominant mode of case 1 was 46,XY,t(3,13),–11,+mar,+mar. One marker chromosome was submetacentric, A₁-sized and had two central bands on the long arms. The other marker was subacrocentric, B-sized and had two proximal bands and one distal band. On day 11 of culture, 8% of the metaphases had 47 chromosomes, the extra chromosome being a No. 21. Otherwise the chromosomal findings of case 1 on days 0 and 11 were very similar, particularly with regard to the translocation and the marker chromosomes which were found in 100% of metaphases. Polyploid metaphases revealed two or three sets of the marker chromosomes. Identity of the translocation chromosome and the marker chromosomes in both preparations was confirmed by their G-banding patterns. The chromosome preparation from a lymph node, removed immediately after death, yielded only one analysable meta-

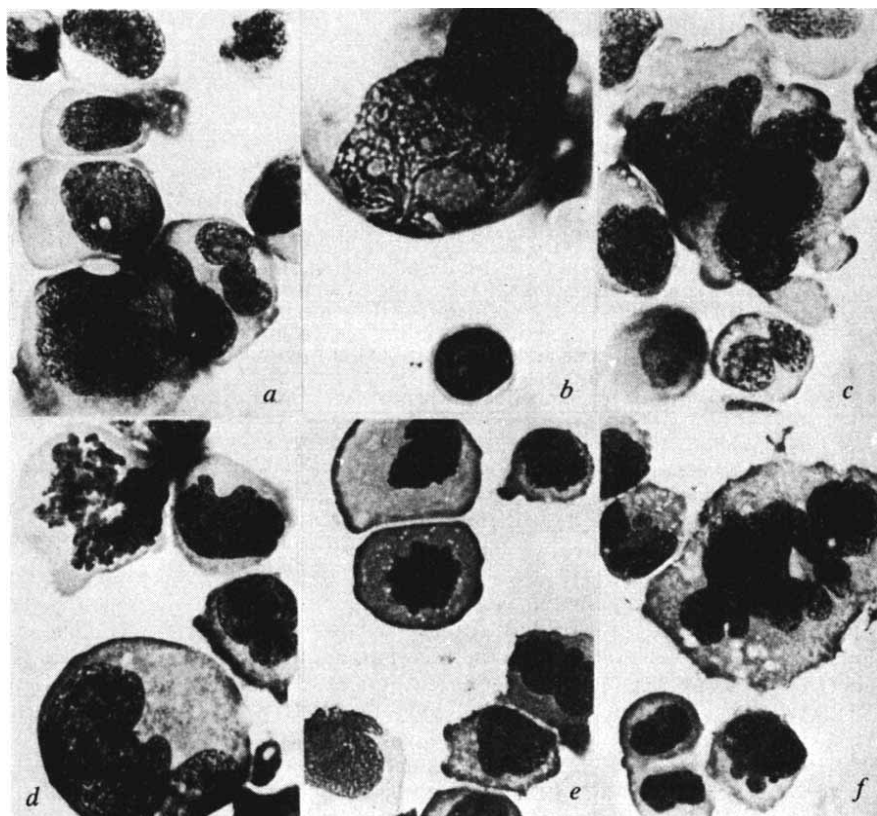


Fig. 1 Composite micrograph showing Hodgkin (HC) and Reed–Sternberg cells (RSC) of case 2. HC and RSC from the original pleural effusion (a–c) and after 112 days in diffusion chamber culture (d–f). Note normal lymphocyte in comparison to HC (b) and mitotic figures of HC (d–f). Original magnification on 24 × 36 mm negative film × 400 (a, c, d–f) and × 630 (b). May–Grünwald–Giemsa stain.

Table 1 Summary of numerical chromosomal findings of cases 1 and 2

No. of metaphases counted	Day 0		Day 11		Day 50	
	Case 1 255	Case 2 100	Case 1 50	Case 2 50	Case 1 0	Case 2 100
No. of chromosomes per metaphase						
46	86.0%	0	84.0%	0	—	0
47	0	0	8.0%	0	—	0
55-77	4.4%	93.0%	0	100.0%	—	95.0%
82-92	6.6%	0	8.0%	0	—	0
110-130	2.2%	6.0%	0	0	—	4.0%
174-186	0.8%	1.0%	0	0	—	1.0%

phase which, however, clearly demonstrated the translocation and the marker chromosomes described above.

In case 2, G banding revealed up to 17 marker chromosomes in the near triploid metaphases, the origin of most of which remained obscure. The most prominent and consistent among the marker chromosomes were a large submetacentric and two slightly different subacrocentric chromosomes. Some metaphases showed a 1q- and a 6q-. All metaphases had supernumerary chromosomes 19 and one or more missing 13. Karyotypic characteristics of the near triploid metaphases were invariably demonstrable in duplicate or triplicate in the pentaploid or octoploid metaphases. With regard to distribution of chromosome numbers, karyotypic characteristics and banding patterns, the results of chromosome analyses on days 11 and 50 in culture were in agreement with those obtained on day 0.

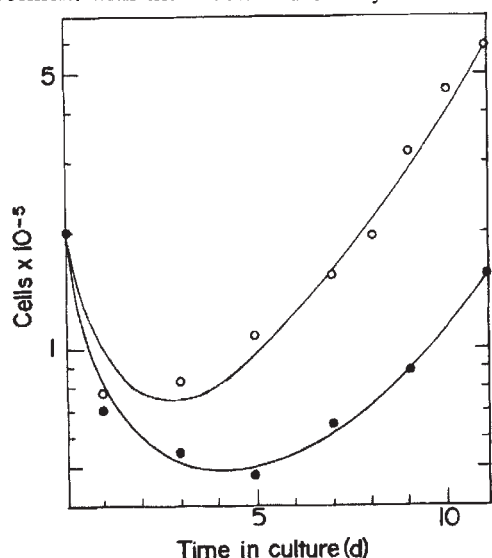


Fig. 2 Hodgkin cells obtained from the pleural effusion of two patients with terminal Hodgkin's disease were cultured in diffusion chambers which were implanted into the peritoneum of irradiated NMRI mice (800 r. ^{60}Co 1 d before chamber implantation), and reimplanted on day 8. Changes in total cell number are shown as a function of time. Symbols represent mean cell number of six to eight diffusion chambers. ●, Case 1; ○, case 2.

On days 0 and 50 the chromosome numbers of 400 further metaphases were estimated at low magnification. The results substantiated the distribution of ploidy values given in Table 1. No normal metaphase could be found at any time in either case.

Cell surface immunoglobulins were determined as before, using an antiserum prepared in rabbits against human immunoglobulins G, A and M $^{\circ}$. Indirect immunofluorescence tests showed a bright membrane fluorescence in 86% (case 1) and 79% (case 2) of the Hodgkin cells and the Reed-Sternberg cells. We looked at 300 cells of case 1 and 200 cells of case 2. This fluorescence was demonstrated both on the cells directly obtained from the effusions and on the cells collected from the chambers after 50 d of culture. It is

thus less likely that immunoglobulins bound secondarily to the cell surface were detected. Spontaneous rosette formation with sheep red blood cells was studied by standard techniques $^{\circ}$. Neither the Hodgkin nor the Reed-Sternberg cells were positive.

Our findings show that the Hodgkin cells we investigated are capable of continuous monoclonal growth. When cultured in diffusion chambers their growth is exponential, with doubling times of 48-72 h. Chromosome analyses suggest that diploid and triploid Hodgkin cells are precursor cells of the polyploid Reed-Sternberg cells. The demonstration of immunoglobulins on the cell surface even after weeks in culture and the absence of rosette formation point to a B-cell origin of both Hodgkin and Reed-Sternberg cells.

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Absence of infectious Epstein-Barr virus in blood in acute infectious mononucleosis

CULTURES of peripheral leukocytes from patients with infectious mononucleosis (IM) readily give rise to B lymphoblastoid cell lines containing Epstein-Barr virus genome. Recent experiments have shown that such lines arise not through the direct outgrowth of cells infected by EB virus *in vivo* but as a result of the transformation *in vitro* of previously uninfected B lymphocytes by infectious virus detectable in the cultures at an early stage 1,2 . For in co-cultures of IM leukocytes and an equal number of foetal leukocytes, the cell lines which emerged were exclusively of foetal origin in 6 out of 16 cases and of mixed origin with foetal cells frequently predominating in the remaining 10 cases; yet foetal cells cultured alone never gave rise to lines 1 . More important, the inclusion of neutralising anti-serum to EB virus in the medium inhibited the establish-

ment of cell lines both in cocultures of IM and foetal cells and in cultures of IM cells alone^{1,2}.

The source of the infectious virus was not established absolutely, and one obvious possibility was that it had come directly from the blood of the acute IM patients where infectious particles could already have been circulating either free in the plasma or sequestered in leukocytes. The present experiments argue against this and support the idea that infectious virus originates from a small number of peripheral IM lymphocytes which carry the EB viral genome in a non-infectious form *in vivo* but which are activated to a cycle of virus production *in vitro*.

Heparinised blood samples (30 to 60 ml) were taken from seven patients with acute heterophile antibody-positive IM between 1 and 4 weeks after the onset of clinical symptoms; serum samples were taken at the same time to investigate the EB viral antibody status of each patient as regards neutralising activity¹, anti-viral capsid antigen (VCA) titre³, and anti-nuclear antigen (EBNA) titre⁴.

About 5 ml of each blood sample was immediately centrifuged at 1,000g for 10 min and the supernatant

then shaken with fresh foetal cord blood leukocytes for 60 min after which the foetal cells were washed, set up in microtestplate cultures, and observed for 8 weeks for evidence of transformation. The techniques used have already been described¹.

The incidence of transformation of the foetal leukocytes after exposure to the various preparations is shown in Table 1. No transformation occurred in any culture of foetal cells exposed to IM plasma or extracts of fresh IM mononuclear cells (day 0 extracts). In contrast, five out of seven IM mononuclear cell extracts prepared after 3 d of culture (day 3 extracts) showed detectable leukocyte-transforming ability; this ability obviously depended on the presence of infectious EB virus since it was only manifest with those extracts pretreated with negative serum and was abolished by pretreatment with neutralising antiserum to the virus. Confirmation of the role of EB virus in the transformation was provided by the finding of EBNA⁵ expression in the transformed cells.

Table 2 shows the incidence of, and mean time to, transformation in the cultures of washed mononuclear cells

Table 1 Incidence of transformation in cultures of foetal leukocytes exposed to plasma and to extracts of mononuclear cells from acute IM patients

Patient no.	Plasma preincubated with		Day 0 extract preincubated with		Day 3 extract preincubated with	
	EB virus negative serum	Antiserum to EB virus	EB virus negative serum	Antiserum to EB virus	EB virus negative serum	Antiserum to EB virus
IM71	0/8	0/8	0/8	0/8	1/8	0/8
IM72	0/8	0/8	0/8	0/8	2/8	0/8
IM74	0/8	0/8	0/8	0/8	6/8	0/8
IM76	0/8	0/8	0/8	0/8	0/8	0/8
IM92	0/8	0/8	0/8	0/8	2/8	0/8
IM94	0/8	0/8	0/8	0/8	0/8	0/8
IM96	0/8	0/8	0/8	0/8	3/8	0/8

plasma was rapidly frozen, and stored at -70°C . The remaining blood was centrifuged on Ficoll-isopaque, and the mononuclear cells were collected and washed as previously described¹, and then divided into three samples for the following treatments: (1) Some washed mononuclear cells were suspended in medium (Eagle's minimum essential with 15% foetal calf serum), cultured in plastic vials at a concentration of 2×10^6 to 3×10^6 cells ml^{-1} and observed regularly for 8 weeks for evidence of transformation, as in earlier work¹. (2) Half the remaining washed mononuclear cells were used to prepare an extract (day 0 extract); the cells were suspended in the medium at a concentration of 1.5×10^8 to 2×10^8 ml^{-1} , disrupted by three cycles of rapid freezing and thawing, and centrifuged at 5,000g for 20 min to deposit the debris, after which the supernatant was collected and stored at -70°C . (3) The rest of the washed cells were cultured for 3 d and were then similarly extracted (day 3 extract) in an equal volume of medium to that used on day 0.

The plasma and the two cell extracts from each patient were assessed for their ability to transform foetal leukocytes in culture. Samples of each preparation were incubated for 60 min at 37°C with either antiserum to EB virus of known neutralising activity or negative serum; the mixtures were

from each patient. It is of interest that those mononuclear cell populations which showed the highest and earliest incidence of transformation when cultured on their own (Table 2) were just those which produced infectious EB virus in culture by the time they were extracted on day 3 (Table 1); conversely the two mononuclear cell samples whose day 3 extracts lacked detectable transforming ability also showed the lowest incidence of transformation when cultured alone.

This correlation confirms and extends similar observations in earlier experiments¹. Table 2 also shows that by the time the patients were investigated in the present study all seven had developed low, but detectable, neutralising antibodies to EB virus, seven had antibodies to VCA, and two had low anti-EBNA titres, but there is no obvious relationship between the serological status of the patients and the behaviour of their mononuclear cells in culture. Nevertheless, the range of serological findings among these seven IM patients is in agreement with results from larger studies^{4,6}.

Thus, the present experiments show that IM patients with clinically manifest disease do not have infectious EB virus in the circulation. At this stage infectious virus is not sequestered in some protected site within peripheral lympho-

Table 2 Acute IM patients: transformation in mononuclear cell cultures and anti-EB virus titres in sera

Patient no.	Transformation		Neutralising	Serum titre Anti-VCA	Anti-EBNA
	Incidence	Mean time (weeks)			
IM 71	4/6	4.5	1:10	1:80	1:10
IM 72	6/6	3.3	1:5	1:80	< 1:2
IM 74	4/6	3.5	1:5	1:80	< 1:2
IM 76	0/6	—	1:20	1:160	< 1:2
IM 92	6/6	4.2	1:20	1:320	1:10
IM 94	2/6	5.0	1:5	1:80	< 1:2
IM 96	6/6	2.8	1:5	1:80	< 1:2

cytes nor is it present as a viraemia, although the latter must obviously occur during prodromal phases before neutralising antibodies develop.

It seems clear therefore that the origin of the EB virus particles present early in cultures of IM mononuclear cells (Table 1, day 3 extracts and ref. 1) and responsible for their transformation to give cell lines, must be peripheral lymphocytes carrying the viral genome in a non-infectious form *in vivo*. These cells when placed *in vitro* do not have the inherent capacity to grow directly into lymphoblastoid lines but are activated into a cycle of virus production; it is this virus which is crucial for the initiation of cell lines since the whole process is inhibited in the presence of neutralising antiserum^{1,2}. Peripheral cells in IM must therefore carry EB virus in a different form of infection from that of the malignant cells in Burkitt's lymphoma, where the presence of the viral genome is associated with a ready ability to grow out directly *in vitro* to give a lymphoma cell line⁷⁻⁹.

It has been suggested¹⁰ that the type of EB viral infection shown by circulating cells in IM may be closer to the latent infections established in ganglia by herpes simplex¹¹ and varicella-zoster¹² viruses, where the viral genome can remain apparently unexpressed for long periods but can be activated into a cycle of virus production by a variety of stimuli including placing the ganglia in culture¹³.

The presence of infectious EB virus in the throat washings of infected individuals^{14,15} (whether primary infection was not apparent, or associated with IM) shows that there is a site of virus replication within the oropharynx. It may be that virus-infected lymphocytes leaving this site and entering the circulation have their productive cycle of virus replication repressed in some as yet unexplained way by the combined actions of the cell-mediated and humoral immune responses which are mounted against EB virus-related antigens. Cells containing EB virus genome, scattered through the body's lymphoid tissues *in vivo* and capable of activation to a virus-productive cycle *in vitro*, would explain why lymphoblastoid lines, closely resembling those produced by the *in vitro* transformation of foetal cells but quite distinct from those of Burkitt lymphoma origin^{16,17}, are obtainable when lymphocytes are cultured from any lymphoma-free individual who is seropositive for EB virus¹⁸.

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Temporary presence of self-reactive cytotoxic T lymphocytes during murine lymphocytic choriomeningitis

MICE injected with lymphocytic choriomeningitis (LCM) virus generate specifically sensitised thymus-derived (T) lymphocytes¹⁻³. Such cells have been shown^{1,2,4} *in vitro* ⁵¹Cr-release assays to be cytotoxic to target cells infected with LCM virus. The specificity of the cytotoxic reaction has been inferred by the observation that the cytotoxic T lymphocytes (CTL) do not lyse noninfected target cells. Moreover, there is evidence that the activity of the CTLs generated is restricted to those virus infected target cells which are compatible with the CTLs in regard to the major histocompatibility complex (MHC)⁵. This peculiar restriction prompted us to study the specificity of CTLs generated in murine LCM. We observed that 4-6 d after infection with the LCM virus the CTLs generated are specifically cytotoxic to syngeneic target cells, irrespective of whether or not they are infected with LCM virus. On the other hand, as the infection proceeds in time, T-cell mediated cytotoxicity is increasingly restricted to virus-infected syngeneic cells. The results imply that during the early phase after infection, murine LCM may represent an autoimmune phenomenon.

We used strain WE-3 (ref. 6) of the LCM virus. The immunisation regime, the preparation of cells, the characteristics of the cells mediating cytotoxicity and the ⁵¹Cr-cytotoxicity assay

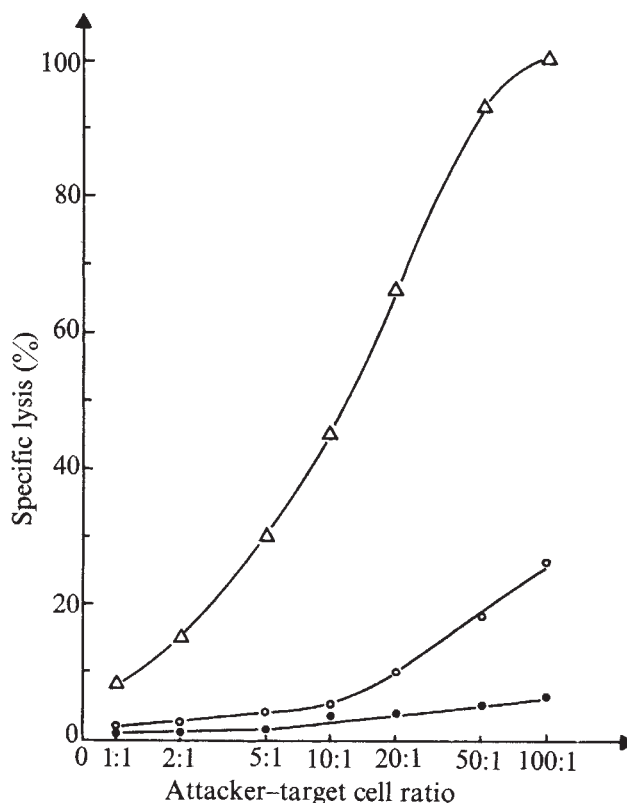


Fig. 1 Dose-response relationship between specific lysis (%) and the ratio of attacker cells to target cells. Spleen cells from LCM infected CBA mice were used at day 8 and tested for cytotoxicity as described in legend to Fig. 2. Δ — Δ , Infected L-929 fibroblasts (H-2^k); $\circ$ — $\circ$, non-infected L-929 fibroblasts (H-2^k); $\bullet$ — $\bullet$, non-infected BALB/c embryonal fibroblasts (H-2^d). The spontaneous release of target cells ranged from 20 to 30%. Each point represents the mean of specific lysis (%) obtained at the indicated ratio of attacker cells to target cells. Since in all assays the standard deviation (s.d.) was less than 6%, only the mean is given.

Table 1 Presence of 'self-reactive' CTLs 6 d after infection of CBA mice with LCM virus

Experiment no.	Cell type	H-2 compatible target cells		Specific lysis (%)		H-2 incompatible target cells	
		Strain	Virus infected	Non-infected	Cell type	Strain	Non-infected
140	L 929 Fibroblast	C3H	92	44	Embryonal fibroblast	BALB/c	10
142			60	35		BALB/c	5
159			58	73		C57BL/6	23
193			89	43			
159	Embryonal fibroblast	CBA	54	50	Macrophage	C57BL/6	4
163			52	32		BALB/c	14
167			57	31		C57BL/6	0
158	Macrophage	CBA	17	16			
142			56	29		BALB/c	5

The cytotoxicity of CBA mice was tested at day 6 after intraperitoneal injection of 1,000 LD₅₀ of LCM virus. Each value represents the mean of triplicate determinations obtained at an attacker: target cell ratio of 20:1 (18 h ⁵¹Cr-release assay). Standard deviation of the data given was less than 6%. Spontaneous ⁵¹Cr-release ranged from 18 to 25% for L 929 cells, and from 20 to 35% for fibroblasts and macrophages.

have been described by Pfizenmaier *et al.*⁷ In brief, virus-infected or normal target cells (peritoneal macrophages, embryonal fibroblasts or L 929 cells) were labelled with ⁵¹Cr-chromate and incubated with immune or normal spleen cells for 18 h. The cytotoxicity assays were performed in triplicates. For each cytotoxic lymphocyte population to be tested, a dose-response curve (Fig. 1) was produced. The percentage specific lysis was calculated as described by Wagner⁸. That the detected cytotoxicity was mediated by T lymphocytes is indicated by the fact that pretreatment of the cells with anti-Thy 1.2 serum plus complement abolished their cytotoxic activity.

Splenic T lymphocytes of CBA mice infected intraperitoneally with 1,000 LD₅₀ LCM virus of the strain WE-3, developed cytotoxic activity against virus infected H-2 identical fibroblast detectable *in vitro* 4–16 d later. Maximal cytotoxicity was detected at day 7–8. H-2 different (allogeneic) target cells were not lysed (Fig. 2). At the peak of the response (8 d after infection) (Fig. 1), the cytotoxic activity generated seems to have been restricted to virus infected target cells compatible with the attacker cells on either the H-2K or H-2D subregion of the MHC. Virus infected target cells compatible on the I-region of the MHC were not lysed (Table 2). The results are in agreement with the work of Doherty and Zinkernagel⁹ and suggest that the target for CTL (as generated at day 8 of LCM) is either closely linked to the phenotypical products coded by the H-2K or H-2D subregion of the MHC, or alternatively, represents serologically defined (SD) 'self' antigens which have been altered in the course of the virus infection⁶.

Figure 1 demonstrates that the CTLs generated (at day 8 after infection) are also cytotoxic, albeit much less effectively against non-virus infected H-2 identical target cells. Although this finding was first explained by invoking cross reactivity of the target cells, we studied the specificity of the CTLs in more detail. Surprisingly, we found that CTLs generated during the early phase of LCM were unable to discriminate between virus infected and non-infected H-2 identical target cells; that is, both cell types were lysed equally well (Fig. 2). Yet allogeneic, H-2 incompatible target cells were not lysed above the usual background. Moreover, the existence of 'self-reactive' CTLs seemed to be transient and restricted to the first 3 d of the

development of cytotoxic activity. In addition, the data given in Table 1 clearly demonstrate that during the early phase of the LCM disease, the generated CTLs lysed not only the syngeneic L cells which were not infected, but also non-infected syngeneic fibroblasts and macrophages. The results therefore suggested, that the 'self-reactivity' can be detected both with L cells, syngeneic fibroblasts and syngeneic macrophages.

On the other hand, the CTLs obtained at the peak of the

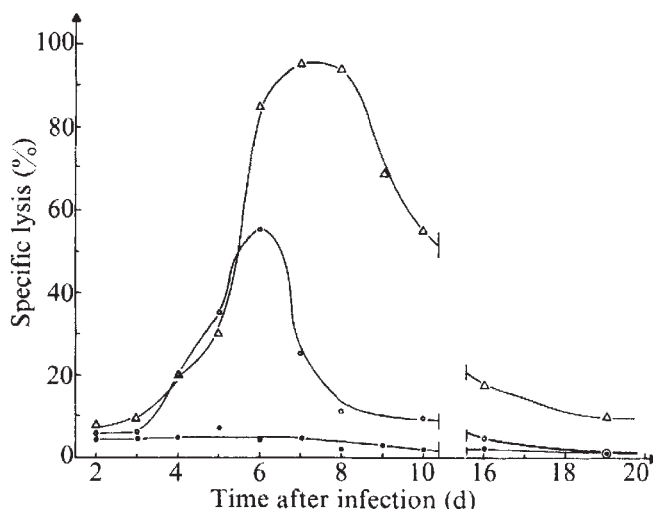


Fig. 2 Kinetics and specificity of cytotoxic T lymphocytes generated during murine LCM. CBA mice were infected intraperitoneally with 1,000 LD₅₀ LCM strain WE-3 and at the given time intervals, spleen cells from 3 mice were pooled and tested at different ratios with 5×10^4 target cells in an 18 h ⁵¹Cr-release test. Each point represents the mean of specific lysis (%) obtained in triplicate determinations at a ratio of 20 attacker cells to one target cell. Since in all assays the standard deviation (s.d.) was less than 6%, only the mean is given. Similar results were obtained in 3 independent experiments. Δ — Δ , Infected L-929 fibroblasts (H-2^k); $\circ$ — $\circ$, non-infected L-929 fibroblasts (H-2^k); $\bullet$ — $\bullet$, non-infected BALB/c embryonal fibroblasts (H-2^d). Spontaneous release ranged from 20 to 30%.

Table 2 Specificity of CTLs generated during LCM (8 d after infection)

Attacker cells derived from*	Lysis of infected target cells (macrophages) (%)				A.TL (H-2 ^u) s kkk d
	CBA/J (H-2 ^k) k kkk k†	DBA/2 (H-2 ^d) d ddd d	B10.A (4R) (H-2 ^{b4}) k kbb b		
CBA/J (H-2 ^k)	60	7	ND‡		ND
B10.BR (H-2 ^k)	ND	ND	74		13
BALB/c (H-2 ^d)	4	45	12		84

*3 mice per group were infected intraperitoneally with 1,000 LD₅₀ of LCM virus strain WE-3. After 8 d the spleen cells were tested for cytotoxicity in an 18 h ⁵¹Cr-release assay. The results given represent the mean specific lysis (%) obtained in triplicate determinations at a ratio of 20 attacker cells to one target cell. The standard deviation (s.d.) was less than 6%. Lysis of non-infected cells ranged from 3 to 10%.

†Indicates the haplotype of the MHC, that is, the H-2K, IA, IB, IC, and H-2D subregion, respectively.

‡ND, Not done.

response (day 7–8) were found to be maximally cytotoxic for H-2 identical target cells infected by the virus. At that point in time a very limited amount of anti-'self' cytotoxic reactivity was detected in the spleen of infected mice (Figs 1 and 2). That the cells mediating the anti-'self' cytotoxicity during the early stage of LCM were T cells in type was suggested by the fact that their cytotoxic activity was abolished after treatment with anti-Thy 1.2 serum plus complement (K.P., unpublished).

In conclusion, on the basis of the specificity of the generated CTLs two phases may be distinguished during murine LCM. On the one hand there is an early phase, characterised by the existence of both anti-self-reactive CTLs and by cytotoxic activity against virus-infected, H-2 identical target cells. On the other hand, at the time of maximal cytotoxicity (7–8 d after infection) the CTLs generated exhibit cytotoxic activity restricted against virus-infected, H-2 identical target cells. In control experiments we tested whether the lysis obtained in the case of non-infected target cells was attributable to an LCM-virus infection of the target cells by virus released from the effector cell population. Since, however, addition of LCM-virus to a mixture of effector cells (obtained at day 8) and non-infected syngeneic target cells did not result in lysis of the target cells during the 18 h assay time (K.P., unpublished) that possibility seems to be ruled out. Whether the CTLs generated during the early stage of LCM can be separated into those reactive against virus-infected, H-2 identical cells, and those reactive against self antigens or, alternatively, whether the development of a more stringent restriction of the cytotoxic activity reflects a maturation of the CTLs to be generated, is under investigation.

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Evidence against a dimeric structure for membrane-bound HLA antigens

HUMAN histocompatibility (HLA) antigens are expressed on the surfaces of almost all cell types and according to various criteria are integral membrane glycoproteins¹. They are composed of equimolar amounts of two dissimilar polypeptide chains which are non-covalently linked and which have molecular weights of about 43,000 and 12,000 as judged by polyacrylamide gel electrophoresis in sodium dodecyl sulphate (SDS)^{2–4}. The larger chain carries the polymorphic allogeneic specificities and all of the carbohydrate². The smaller chain represents β_2 -microglobulin, whose amino acid sequence shows marked homology to the C_H3-domain of the γ -1 chain of immunoglobulin G (ref. 5). This homology and other arguments have led to the proposals that HLA antigens and immunoglobulins have a common evolutionary origin^{6,7}, and that HLA antigens have an immunoglobulin-like structure

in which two basic units of one each of the larger and smaller polypeptide chains are linked either non-covalently⁸ or by a disulphide bridge^{9,10} to give a 4-chain dimer of molecular weight 110,000. In contrast to the latter proposal, some results presented here suggest that HLA antigens do not have a dimeric structure.

HLA activities were measured by inhibition of cytotoxicity¹¹ using peripheral blood lymphocytes with sera 3002X, P2692B and P3812B for HLA-A2, sera 1209F, 1014I and P1595B for HLA-A1, sera P1422B and P1538B for HLA-B8, and sera P1040B and P3428B for HLA-B13. A highly purified preparation of the plasma membrane of the lymphoblastoid cell-line BRI 8 (HLA type: A1, A2, B8, B13, 4a and 4b) (ref. 1), and of the plasma membrane fraction of a human spleen prepared by a modification of a method described previously¹² (HLA type: A2, A3, BW35) were used as sources of the whole HLA antigens. More than 86% of the plasma membrane HLA activity were solubilised by 1% Na deoxycholate (pH 8.2) as judged after centrifuging at 100,000g for 1 h.

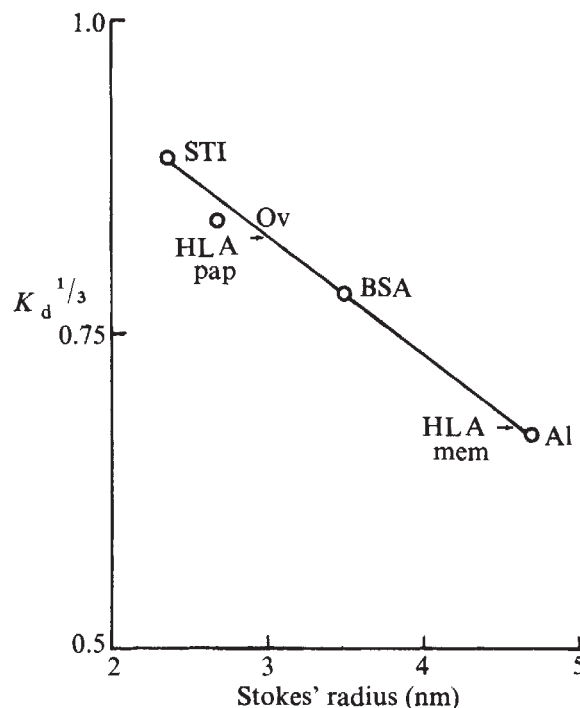


Fig. 1 Relationship between Stokes radii and elution positions ($K_d^{1/3}$) of water-soluble globular proteins from a column of Ultragel AcA 34 in 0.5% sodium deoxycholate. $K_d^{1/3}$ was calculated as previously described¹³. The Stokes radii were those reported by Andrews¹⁴ except for that of soybean trypsin inhibitor which was calculated from the diffusion coefficient and molecular weight¹⁵. AI, aldolase; BSA, bovine serum albumin; Ov, ovalbumin; STI, soybean trypsin inhibitor. The arrows indicate the measured $K_d^{1/3}$ for the whole HLA antigens (mem) and for the papain-solubilised HLA antigens (pap). Experimental details are given in the legend to Fig. 2.

The molecular nature of HLA antigens was adduced from their gel-filtration behaviour and measurements of their $S_{20,w}$ (ref. 1) and Stokes radii (Fig. 1) in the presence of sodium deoxycholate. Gel filtration of the deoxycholate-solubilised BRI 8 plasma membrane on a column of Ultragel AcA 34 gave (Fig. 2) a single peak of HLA-A2 activity. HLA activities A1, B8 and B13 of the BRI 8 plasma membrane and the HLA antigens of the plasma membrane fraction from the human spleen occupied identical positions with that shown for HLA-A2. Similar elution patterns were also obtained using Sephadex G-150 and G-200, and Sepharose 6B. In each case, the eluted peak accounted for > 86% of the HLA activity added to the column, and no larger aggregates or gross polydispersity was detected. The absence of aggregates in our experiments is in marked contrast with a recent report¹⁰ on a

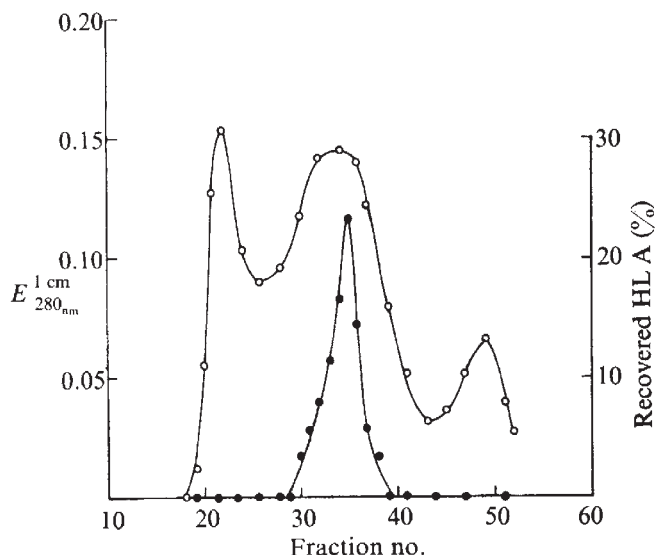


Fig. 2 Gel filtration of deoxycholate-solubilised BRI 8 plasma membrane on Ultragel AcA 34. BRI 8 plasma membrane containing 4 mg of protein was incubated for 30 min at room temperature with 1 ml of 1% sodium deoxycholate in 10 mM Tris-HCl buffer (pH 8.2) and centrifuged at 100,000g for 1 h. The supernatant (90% of the protein; 86% of the HLA activity) was eluted from a column (91 × 1.6 cm) of Ultragel AcA 34 (LKB Instruments Ltd) with 0.5% sodium deoxycholate at a flow rate of 4.9 ml h⁻¹. Fractions (2.45 ml) were assayed for protein (○; $E_{280\text{nm}}^{1\text{cm}}$) and HLA-A2 activity (●). HLA activities A1, B8 and B13 occupied an identical position with that illustrated for HLA-A2. The HLA antigens of the deoxycholate-solubilised plasma membrane from human spleen gave the same elution pattern.

crude membrane preparation from a lymphoblastoid cell line. In that experiment about 40% of the HLA activity was eluted in a second peak, which was provisionally identified as dimer, as well as some larger multimers (see Fig. 3 in ref. 10). The reason for this difference is not known. It is, however, unlikely to be due merely to the use of different cell lines since no aggregates were detected in the current study with human spleen. The main differences between the two studies concern the state of the cells used for membrane preparation and the quality of the membrane fraction. In the present experiments the cells were used immediately after growth (viability > 80%) and the plasma membrane was extensively purified to separate it from endoplasmic reticulum¹. Furthermore, the HLA antigens were not knowingly subjected to reducing conditions during cell breakage and processing of the plasma membrane. It is possible that Cresswell and Dawson's elution pattern¹⁰ reflects aggregation through disulphide interchange. This aggregation is promoted by storage (compare the elution pattern of an aged sample of human serum albumin¹⁶). Also, the crude membrane preparations used probably contained endoplasmic reticulum which is rich in an enzyme catalysing sulphhydryl-disulphide interchange in various proteins¹⁷.

The molecular sizes and shapes of the HLA antigens in sodium deoxycholate are summarised in Table 1. The values are notable for various reasons. First, the molecular weights of the papain-solubilised HLA antigens in deoxycholate (46,000) agreed closely with those calculated from the values for the β_2 -microglobulin and HLA polypeptide chains measured by SDS-polyacrylamide gel electrophoresis (about 12,000 and 34,000, respectively⁹).

Second, the molecular weights of the deoxycholate-solubilised A and B series antigens (88,000 and 78,000 respectively) were larger than those predicted from the sizes of the β_2 -microglobulin and HLA polypeptide chains determined by SDS-polyacrylamide gel electrophoresis (about 55,000 including β_2 -microglobulin⁹), but were not sufficiently large to accommodate a disulphide-linked dimer. The most likely explanation

for this difference is that, as suggested previously¹, HLA antigens resemble other integral membrane proteins (for example cytochrome b_5 ; ref. 18) in possessing a hydrophobic domain which binds non-ionic and weakly anionic detergents^{19,20}. As a result the deoxycholate-solubilised HLA antigens will possess anomalously high molecular weights when assessed relative to water-soluble globular proteins. This anomaly will not, however, apply to estimates of the molecular sizes of the papain-solubilised antigens, because of the removal of the hydrophobic domain during proteolysis and the consequent loss of detergent binding. If it is assumed that the true molecular weight of HLA antigens is 55,000 (as determined by polyacrylamide gel electrophoresis⁹) and that the difference between this molecular weight and those reported for the whole antigens in Table 1 is entirely due to bound detergent, then the A and B series antigens bind 0.57 and 0.39 g of deoxycholate per g of protein respectively. These values are in accord with those reported for various membrane proteins (0.29–0.64 g per g of protein)²⁰ and with those estimated by equilibrium dialysis for two fractions of BRI 8 plasma membrane containing about 3 and 15% of HLA antigens (0.68–0.72 g per g of protein; D. Snary, unpublished). Detergent binding is also consistent with the higher molecular weights reported for HLA and H-2 antigens (460,000 and 380,000, respectively) in different non-ionic detergents (Brij 99 and Nonidet P40 respectively)^{3,21}, since these probably express the higher micelle sizes of the bound detergent.

Alternatively, the larger molecular weight of the deoxycholate-solubilised HLA antigens could accommodate a non-covalently bonded dimer⁸ if deoxycholate caused dissociation. This is considered improbable primarily because comparisons of the gel-filtration patterns of haemoglobin on Sephadex G-200 in the presence and absence of 1% Na deoxycholate failed to reveal any evidence for dissociation. This result is especially significant since haemoglobin is already partly dissociated in aqueous solution and the majority of the interactions between the α_1 and β_2 subunits are non-polar²² and would be expected to be particularly sensitive to dissociation by detergent.

Third, the apparent difference between the molecular weights of the deoxycholate-solubilised A and B series antigens (Table 1) probably arises from a larger amount of deoxycholate binding to the A antigens than to the B antigens. This variation may reflect a difference in hydrophobicity in which case it may indicate that the A series antigens are inserted more deeply into the lipid bilayer than B series antigens.

Fourth, estimates of the frictional ratios¹³ of the A and B antigens gave 1.49 and 1.55 respectively. Although these values suggest that the membrane-bound antigens are moderately

Table 1 Molecular size and shape of HLA antigens in sodium deoxycholate

HLA antigens	Antigen series	$S_{20,w}$	Stokes radius (nm)	Molecular weight	f/f_0
Na deoxycholate-solubilised	A	5.15	44.0	88,000	1.49
	B	4.55	44.0	78,000	1.55
Papain-solubilised	A	3.68	29.8	46,000	1.26
	B				

$S_{20,w}$ values were determined previously by sucrose density gradient centrifugation in 1% sodium deoxycholate (see Table 3 in ref. 1). The Stokes radii were measured by gel filtration on a column of Ultragel AcA 34 that had been standardised using water-soluble globular proteins in 0.5% sodium deoxycholate (Fig. 1). Molecular weights were calculated from $S_{20,w}$ and Stokes radii (a) by using the relationship molecular weight = $6\pi N\eta S/1 - \rho\bar{v}$ (ref. 13), where N is Avogadro's number, η the viscosity and ρ the density of water at 20 °C. The partial specific volume ($\bar{v}$) for the papain-solubilised HLA antigens was taken to be 0.72, whereas that for the deoxycholate-solubilised whole antigens was assumed to be 0.74 on the basis that they contain 8% carbohydrate (E. Appella, personal communication) and bind 0.5 g of deoxycholate per g of protein (see text). The frictional ratio (f/f_0) was calculated according to ref. 13.

asymmetrical, the possibility that the asymmetry is primarily due to the association of the bound detergent with one region of the molecule only cannot be ruled out at present.

In contrast to previous work^{9,10}, no evidence was obtained in the current study for the presence of dimers. It was concluded that the above results cast doubt on the validity of the 4-chain, dimeric, immunoglobulin-like model that has been proposed for membrane-bound HLA antigens, but do not rule out the possibility of a common evolutionary origin for HLA antigens and immunoglobulin.

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Identification of Ss protein as murine C4

DEMANT *et al.*¹ showed that the Ss-Slp region in the H-2 complex in mice affected the level of serum complement; the Ss high mouse having a higher complement activity than the Ss low mouse. They went on to show that antiserum to Ss protein could deplete complement activity from mouse serum, which has been confirmed in another laboratory². This was the first association described between complement and the principal histocompatibility locus. Further associations have since come to light. In the mouse, C3 levels are controlled in part by a gene in the H-2 region³ although the effect is not large. On the other hand, the 'C5 deficiency gene' in the mouse is not linked to H-2 (ref. 4). In man the allotypes of factor B show close linkage to HLA (ref. 5). C2 deficiency in man is also HLA linked in family studies⁶ and a marked linkage disequilibrium has been observed between the 'C2 deficiency gene' and the haplotype HLA10,W18. In the family described with C4 deficiency, HLA linkage has also been described⁷. The C3 allotypes in man, however, are not linked to HLA, nor is the 'C3 deficiency gene'⁸. For guinea pigs, the

'C4 deficiency gene' is linked to the principal histocompatibility locus⁹. Demant *et al.*¹ showed that the Ss protein is likely to be a complement component, and in this communication we provide evidence that the Ss protein is the murine equivalent of the fourth component, C4.

Antiserum to Ss was prepared and rendered monospecific as before¹. Antiserum to mouse C3 was prepared as before¹⁰ and rendered substantially monospecific by absorption with the serum of mice treated with cobra venom factor. A test of total haemolytic activity confirmed the observation that the Ss high mice had almost twice the activity seen in the Ss low mice.

Functional tests for the components that can be measured by standard functional assays for human complement¹¹ were undertaken as shown in Table 1. These results show several components which the Ss protein cannot be. Thus Ss is unlikely to be any of the components C5-C9 since the lytic titre of mouse serum on erythrocyte antibody complexes which reacted with the first four components of complement (EAC 1423) is somewhat higher in the serum of the Ss low mice. Similarly, the Ss protein seems not to be C1 nor either of the two factors of the alternative pathway which are required for reaction with cobra venom factor, that is factor B or factor D. Since human factor B is known to be linked to the HLA locus, the observation that the Ss protein seems not to be factor B is particularly significant. The observation was confirmed by showing that anti-Ss failed to prevent the diffusion of the cobra venom C3-convertase formed on adding cobra venom factor to normal mouse serum. Depletion of factor B does not in any case decrease the total haemolytic titre of human serum (A.M. and P.J.L., unpublished). Functional tests were also carried out for the C1 inhibitor and the C3b inactivator. In neither case were differences found between Ss high and Ss low mice.

Table 1 Functional complement activity in Ss high and Ss low strains of mice

	B 10.D2/N H-2 ^d Ss high (h-a)	B10.BR H-2 ^k Ss low (l-o)
CH50 per ml		
C1 (titre)	8	4
C5 ~ C9 (titre)	1,280	1,280
C1-inhibitor	3	8
(C1 required to react with 100 µl serum)	20 µl	30 µl
C3b-inhibitor (titre)	40	40
(measured by conglutinin activation)		
CVF-cofactors	100%	98%
(factors B + D)		
C3 (antigenically)	158	180

CVF, Cobra venom factor. h-a, Ss^{high} Slp^d; l-o, Ss^{low} Slp^k.

Functional tests for C4, C2 and C3 were difficult to carry out with mouse serum. C3, however, could readily be measured immunochemically and, insofar as a difference was discovered among the mice tested, there was a modest increase in C3 levels in the Ss low mice. This agrees with the findings of Ferreira and Nussenzweig³. To attempt to identify Ss protein as either of the two remaining components—C4 and C2—experiments were carried out using an anti-Ss antiserum to detect Ss protein as a fixed complement component on erythrocytes (Table 2). When fresh mouse serum was added to EA, an intermediate was made that could be agglutinated more strongly and to higher titre with anti-Ss showing that more Ss protein has been fixed. In this experiment a C5-deficient mouse serum was also used to make EAC. This intermediate also was agglutinated (albeit weakly being a Ss low strain) by anti-Ss serum. This confirms that Ss is not a component of the complement sequence at the C5 stage or beyond.

When the EAC made with the Ss high serum was allowed to decay, either in EDTA to remove C1 as well as C2, or in half-ionic strength complement buffer to remove C2 only,

Table 2 Assessment of Ss as agglutinin on complement-coated cells

Ss status C5 status Agglutination titre with Anti-C3 Anti-Ss		EAC(CBA) l-o +ve	EAC(AKR) l-o -ve	EAC(BALB/c) h-a +ve	EA
		729	729	729	0
		1	27 (weak)	27 (strong)	0
Agglutination titre with anti-Ss					
EAC (BALB/C)	Undecayed		(EAC1423(5))		27
EAC (BALB/C)	Decayed 90 min in CFT-sucrose		(EAC143)		27
EAC (BALB/C)	Decayed 90 min in EDTA-saline		(EAC43)		27
					0
EA	+ NH ₃ treated BALB/c complement				0
EAC (CBA)	+ NH ₃ treated BALB/c complement				1
EA	+ Heated (56 °C, 30 min) BALB/c complement				0

The figures are the highest dilutions of antiserum giving rise to agglutination. CFT, Complement fixation test buffer.

agglutination by anti-Ss was not impaired. This strongly suggests that the Ss protein is C4. A further experiment was performed to confirm this conclusion. EAC was made with Ss low serum and subsequently incubated with NH₃-treated Ss high serum. This conferred on the intermediate no further reactivity with anti-Ss. This experiment indicates that Ss is not C2. The same experiment can also be taken as evidence that Ss is not properdin because activated properdin will attach to fixed C3b (ref. 12).

Table 3 Assessment of Ss by haemagglutination inhibition

Strain	Ss status	Dilution of serum inhibiting agglutination of EAC (BALB/c) by 3 agglutinating doses of anti-Ss
C3H/He/j ♂	l-o	20
CSW ♂	h-a	140
B10.A ♂	h-a	140
B10.D2/n ♂	h-a	240
B10.BR ♂	l-o	20
BALB/c ♂	h-a	140
BALB/c ♀	h-a	80
CBA ♀	l-o	30

Using EAC made with the Ss high serum and three minimum haemagglutinating doses of anti-Ss serum, we could assay the Ss level of several mouse sera by haemagglutination inhibition. Although such experiments are complicated by the presence of immunoconglutinins in individual mouse sera, Table 3 shows that it is possible to get a measure of the concentration of Ss in serum by this technique. This would confirm that the antigen detected by the anti-Ss serum on EAC is the same as that normally measured by precipitation.

These experiments show that the protein reacting with anti-Ss antiserum is a complement component bound firmly and stably to EA at a stage before C5 and that the properties of the Ss 'agglutinin' correspond to what is anticipated for C4.

In view of the probability that the structural genes for several complement components are coded in the H-2 region, the possibility should not be wholly discounted that the method used to raise anti-Ss antisera could raise antibodies to more than one complement protein. It might therefore be preferable to say that murine C4 is an Ss protein rather than necessarily the unique Ss protein.

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H-2 linked Ss protein is C4 component of complement

THE major histocompatibility complex in the mouse encompasses several distinct loci, which, with one exception, control the expression of cell surface glycoproteins. The exception is the Ss-Slp traits¹. The Ss trait involves regulation of the level of a plasma protein, the Ss protein². The S region of the H-2 complex, whose marker gene controls the Ss-protein concentration, also regulates the haemolytic complement level³. This has led to the suggestion that the Ss protein is a component of complement^{3,4}. We have isolated the Ss protein and demonstrate here that it is the murine equivalent of C4.

In our initial attempts to purify the Ss protein we used conventional fractionation procedures. Serum from male BALB/c mice (Ss^h genotype) was initially separated by Sephadex G-200 gel chromatography followed by successive steps of DEAE-Sephadex ion-exchange chromatography (twice), and as the final purification step, recycling chromatography on Sephadex G-200. Ss protein was monitored in chromatographic fractions by immunodiffusion analyses, using a rabbit antiserum against a high molecular weight plasma protein fraction from BALB/c serum. The antiserum was rendered monospecific by repeated absorptions with serum from CBA (Ss^l phenotype) male mice⁵. The isolated protein was homogeneous and of high purity as shown by several physicochemical, chemical and immunological criteria (L.S., in preparation). It consisted of four polypeptide chains, each of apparent molecular weight about 35,000, which were held together in pairs by disulphide bonds. But during the isolation procedure the molecular weight of Ss protein became considerably smaller. Sedimentation-diffusion analyses of the Ss protein present in unfractionated plasma suggested a molecular weight of about 200,000 compared with the 140,000-dalton molecule isolated. It thus seemed likely that either the Ss protein in plasma is linked to another protein, which

was lost during isolation, or is the Ss protein proteolytically degraded during isolation. We therefore tried to improve the isolation by speeding it up. A rabbit antiserum was raised against the 140,000-dalton component. This antiserum reacted with a single component in plasma, as shown by extensive immunodiffusion and immunoelectrophoretic analyses. A radioimmunoassay developed with this antiserum (L.S., in preparation) showed that male mice contained about twice as much of the Ss protein as did female mice, regardless of strain. Furthermore, strains with the Ss^h haplotype had approximately 10 times more of the isolated component in serum than mice of the Ss^i genotype. These data strongly support the view that the antiserum is directed against the Ss protein¹.

The IgG fraction of the anti-Ss-protein serum was coupled covalently to Sepharose 4-B to obtain an immunosorbent column. The Ss protein was isolated from EDTA-plasma, which had first been passed over a column of Sepharose 4-B coupled with aggregated rabbit IgG to remove material sticking nonspecifically to IgG. The breakthrough peak recovered from the aggregated Ig column, which contained all the immunologically active Ss protein as measured by radioimmunoassay, was subsequently passed over the anti-Ss column. The bound material, which was desorbed by lowering the pH of the eluent, was labelled with ¹²⁵I and analysed by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis and by gel chromatography in 6 M guanidine hydrochloride. A single component with an apparent molecular weight of slightly less than 200,000 was obtained from the anti-Ss column. On reduction and alkylation in denaturing conditions, the Ss protein resolved into three (or sometimes four) distinct polypeptide chains. Two of the chains appeared in the 70,000–80,000-dalton region on a column equilibrated with 6 M guanidine hydrochloride. The third chain had an apparent molecular weight of about 23,000 and in some preparations small amounts of a 13,000-dalton component was present. It is thus reasonable to conclude that the Ss protein isolated by conventional techniques most likely represents a proteolytically derived fragment of the larger molecule.

The molecular characteristics of the immunosorbent-purified protein suggested that if indeed it is a component of complement it most likely is C3, C4 or C5, on the reasonable assumption that mouse and human complement components are similar in structure⁵. Since mouse strains

genetically deficient in C5 may have the Ss^h genotype⁶, C5 could be ruled out as the Ss protein. The level of C3 was estimated by single radial immunodiffusion of serum from three strains with the Ss^i genotype and five with the Ss^h genotype. No statistically significant difference in the serum concentration of C3 was found. Although it has been claimed that the level of C3 is regulated in part by the H-2 complex, even in that study no correlation was found between the Ss genotype and the level of C3 (ref. 7).

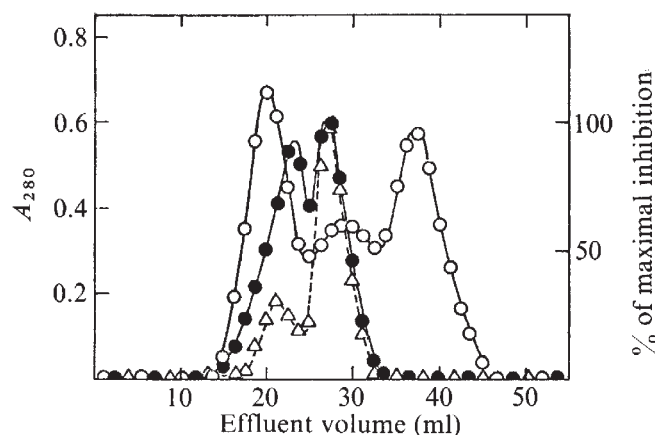
Our efforts were therefore concentrated on the possible relationship between C4 and the Ss protein. Human C4 was isolated⁸ and its molecular characteristics were compared with those of the Ss protein. The human C4 had a molecular weight on SDS-polyacrylamide gel electrophoresis very similar, if not identical, to that of the immunosorbent-purified Ss protein. When human C4 is reduced and alkylated two large polypeptide chains are released. Their molecular weights were indistinguishable from those of the main Ss-protein chains. Furthermore human C4 contained a polypeptide chain with an apparent molecular weight of 35,000 in contrast to the 23,000-dalton component derived from the Ss protein. This discrepancy in the polypeptide chain structure between the two proteins may be genuine, but could have arisen because different isolation procedures were used. It should be remembered that serum contains a C4b inactivator which sequentially cleaves C4 into smaller fragments⁹.

To examine further the relationship between human C4 and the Ss protein, the possibility of immunological cross reactivity was examined. A sensitive radioimmunoassay for human C4 was developed since in immunodiffusion analyses anti-Ss did not precipitate human C4 and vice versa. It could however, be shown that the binding of ¹²⁵I-labelled C4 to a monospecific antiserum against human C4 was almost abolished in the presence of immunosorbent-purified Ss protein. In the Ss protein radioimmunoassay human C4 competed very efficiently with ¹²⁵I-labelled Ss protein for binding to the antibodies. The specificity of the reactions was examined by monitoring the occurrence of the Ss protein in fractions obtained after gel chromatography of mouse serum on a column of Sephadex G-200. Figure 1 shows two peaks of Ss-protein-reacting material produced in the Ss protein radioimmunoassay. The same distribution of material was obtained when antibodies against human C4 were used, and similar patterns were recorded even when ¹²⁵I-labelled human C4 was used. These data strongly suggest that Ss protein is murine C4. The earliest eluted Ss-protein-containing peak is greatly diminished, with a concomitant increase in the amount of Ss protein present in the second peak, in EDTA-plasma. The high molecular weight of the Ss-protein-reacting material in the first peak (sedimentation coefficient about 18S) may represent Ss protein bound to other components, especially since it is known that in fluid phase C423 may occur¹⁰.

To identify the Ss protein unequivocally it must be shown to reconstitute functionally a C4-deficient serum (R4-serum). We have so far failed to do this. The drastic elution conditions that have to be used to desorb the Ss protein from the immunosorbent column may derange the protein so that when it is recovered it is inactive. We were able, however, to test whether Ss-depleted serum was deficient in C4. Figure 2 shows that the material that passed over the anti-Ig column had substantial C4 activity whereas the Ss-protein-depleted fraction was devoid of functional C4 (Fig. 2). This seems to identify the Ss protein functionally with C4.

Lachmann *et al.* in the preceding communication¹¹ examined the relationship between the Ss genotype and various complement components in functional complement assays. Using an antiserum rendered specific for Ss protein, they assessed by absorption the presence of Ss on cells coated with complement. Although using a different

Fig. 1 Gel chromatography of BALB/c male serum on a Sephadex G-200 column (76 × 1 cm) equilibrated with 0.02 M Tris-HCl buffer, pH 8.0, containing 0.15 M NaCl. Total protein was estimated by absorbance at 280 nm (○). The distribution in the effluent of Ss-protein-reacting material was assessed by radioimmunoassay with the 140,000-dalton fragments of the Ss protein as the ¹²⁵I-labelled component and with antiserum against the Ss protein (●) or human C4 (△) as the binding reagent.



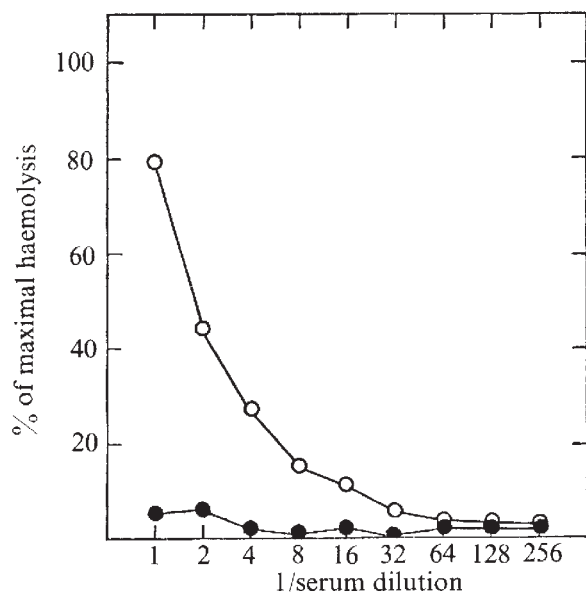


Fig. 2 Reconstitution of the haemolytic complement activity of C4-deficient guinea pig serum with Ig (○) or Ss protein (●) depleted BALB/c male EDTA-plasma. Aliquots of the mouse plasma were depleted of Ig and Ss protein, respectively, by passage over immunosorbent columns containing covalently bound antibodies of the appropriate specificities. The break-through peaks recovered from the immunosorbent fractionations were concentrated separately, dialysed against veronal buffer, and adjusted to the original volume of the plasma sample. Guinea pig serum was depleted of C4 by NH_3 treatment (R4 serum). EA was obtained by mixing sheep red blood cells (SRBC) with the IgM fraction of a rat antiserum against SRBC. Haemolytic complement assay was performed as follows. To 100 μl of R4 serum, diluted 1/20 in gelatin-containing veronal buffer, 100 μl of the relevant mouse plasma fractions, depleted of Ig or Ss protein, at the indicated dilutions, and 50 μl of 1% EA were added. After incubation at 37 °C for 30 min, the mixtures were diluted with 1 ml of cold veronal buffer. The cells were pelleted by centrifugation at 400g for 10 min and the absorbance at 415 nm in the supernatant was estimated. The 100% value in the figure is taken as the absorbance obtained in the complement assay with untreated BALB/c EDTA-plasma. For all values blank readings have been subtracted.

approach than the present, their study demonstrates that the Ss-protein is murine C4.

Our data reinforce the importance of the H-2 region in various immunobiological systems. As well as its role in cell mediated immunity, the major histocompatibility complex now seems to be involved in an effector function of the humoral immunity system. Not only C4 but C2 (ref. 12), C3 (ref. 7) and factor B of the alternate pathway¹³ are regulated by this gene complex. Whether it also controls other components of the complement system remains to be elucidated. We thank Dr P. J. Lachmann for communicating his data before publication. This work was supported by grants from the Swedish Medical Research Council and the Swedish Cancer Society.

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Influence of negative lipids on interactions between artificial membranes and cholinergic drugs

EFFORTS have been made to incorporate "cholinergic systems" into artificial lipid membranes^{1–3}. In all cases, material was obtained from animal tissues and incorporated into artificial systems, cholinergic drugs were added to the bathing solutions and different "permeability changes" were described. The available information on the interactions between pure lipid membrane and cholinergic agents is, however, scarce⁶ and to establish the role of proteins and lipids in the reported findings is difficult. We report here results showing that a negatively charged lipid induces "cholinergic reactivity" in artificial lipid membranes which can be prevented by *d*-tubocurarine.

Planar artificial lipid membranes were made in a septum separating two aqueous phases. Increasing voltages were applied from an external d.c. source and the membrane resistance was calculated from the current–voltage relationship which was constant between 0 and 150 mV. Different salt gradients were then established across the membrane

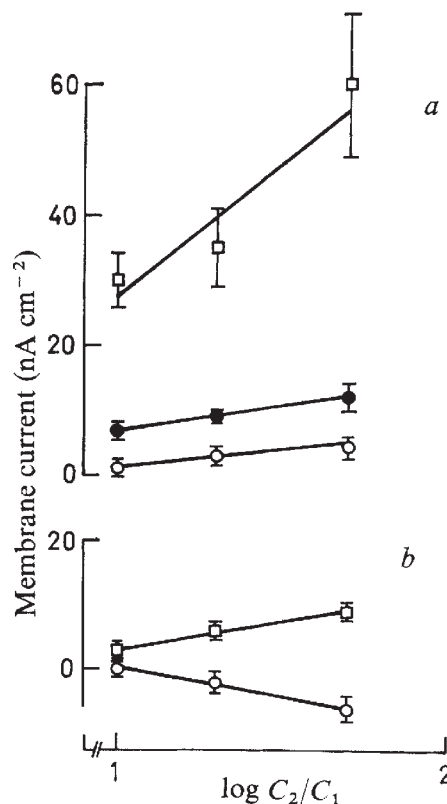


Fig. 1 Membrane current (voltage clamped at 0 mV) as a function of AChCl gradients across the membrane. *a*, Control; *b*, in the presence of *d*-TCCl (10^{-4}M) in the bath. ○, PC plus cholesterol membranes; ●, PC, cholesterol and 1% PS; □, PC, cholesterol + 10% PS.

maintaining the potential clamped at 0 mV. The current flowing through the membrane was recorded and the corresponding membrane potential was calculated using the previously determined resistance. A detailed description of this technique was made elsewhere⁷. The membrane-forming solutions contained 2.3 mg ml⁻¹ synthetic DL- α -dipalmitoyl lecithin (PC) (Sigma, standard for chromatography), 2.3 mg ml⁻¹ synthetic cholesterol (Sigma, 99%) and, in some cases, different amounts of bovine phosphatidyl-L-serine (PS) (Sigma, 95%) dissolved in chloroform-methanol-tetradecane (1.0:0.8:0.5; v/v).

Table 1 Calculated potentials for a tenfold salt gradient across the membrane (mV)

	100% PC	99% PC-1% PS	90% PC-10% PS
AChCl	-1.6 $\pm$ 2.0	-8.4 $\pm$ 1.1	-20.2 $\pm$ 1.3
ChCl	-1.2 $\pm$ 0.6	-4.5 $\pm$ 0.4	-17.9 $\pm$ 1.4
<i>d</i> -TCCl	-8.6 $\pm$ 2.1	-13.6 $\pm$ 2.8	-33.4 $\pm$ 6.1
NaCl	+12.6 $\pm$ 3.5	+7.4 $\pm$ 1.2	-30.1 $\pm$ 2.8

The negative sign indicates cationic selectivity and the positive sign indicates anionic selectivity. Membrane potentials were calculated from membrane current and membrane resistance.

Figure 1a shows the current that flows through the membrane when different acetylcholine chloride (AChCl) (Sigma, preweighed vials, 99%) gradients were established. The membranes were prepared in the presence of 1 mM AChCl solution buffered with 1 mM Tris-PO₄ (pH 7, at room temperature). It can be observed that current intensity is a linear function of the logarithm of the ratio of AChCl concentration in the two chambers. The corresponding membrane potentials are shown in Table 1. The membrane solution used contained as amphipathic molecule only PC, PC plus 1% PS or PC plus 10% PS. It can be observed that as PS concentration increases, membrane current and membrane potential become greater. The sign of the potential indicates cationic selectivity. These results show that, specially in the presence of negative lipids, AChCl gradients at high concentrations can induce cationic membrane potentials showing Nernst-type behaviour. This AChCl potential can be blocked by *d*-tubocurarine (*d*-TCCl). Membranes were made in the presence of 10⁻⁴ M *d*-TCCl (Sigma) and different AChCl gradients were applied. Figures 1a and b compare results with membranes containing only PC or PC plus 10% PS with or without *d*-TCCl in the bath.

Fig. 2 Membrane current (voltage clamped at 0 mV) as a function of the concentrations of different agents on one side. $\circ$, *d*-TCCl; $\bullet$, AChCl; $\square$, NaCl. The membranes were initially made in 1 mM Tris-PO₄ buffer. The *d*-TCCl curve was drawn according to the experimental points. The AChCl and NaCl curves were obtained by moving the *d*-TCCl curve to the right.

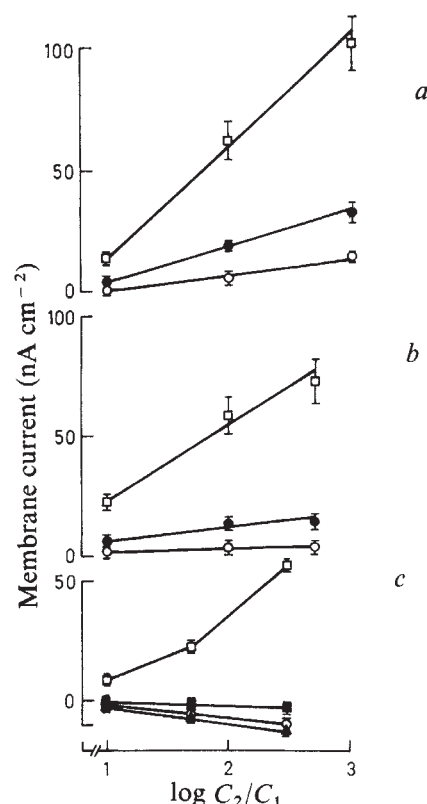
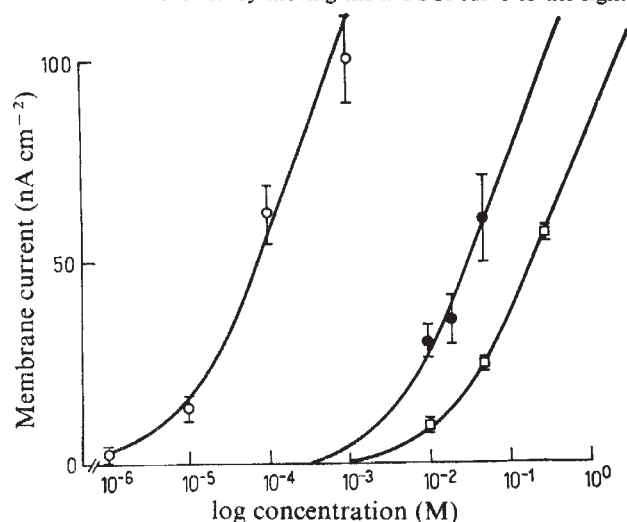


Fig. 3 Membrane current (voltage clamped at 0 mV) as a function of different gradients. $\circ$, PC + cholesterol membranes; $\bullet$, PC, cholesterol and 1% PS; $\square$, PC, cholesterol and 10% PS; $\triangle$, PC + cholesterol in the presence of *d*-TCCl (10⁻⁴ M); $\blacksquare$, PC, cholesterol and 10% PS in the presence of *d*-TCCl (10⁻⁴ M). a, *d*-TCCl gradients; b, ChCl gradients; c, NaCl gradients.

To interpret these results AChCl, *d*-TCCl and NaCl gradients were established across membranes containing different proportions of PS. Membranes were made in 1 mM Tris-PO₄ (pH 7.0) and the tested salts were added to one side of the membrane at increasing concentrations. Figure 2 shows the results obtained. In the presence of 10% PS NaCl, AChCl or *d*-TCCl induce cationic potentials. The concentration ranges in which this phenomenon is apparent are, however, quite different. These results could be explained by a different effective concentration for each one of these species at the membrane interface. Assuming that *d*-TCCl accumulates at the surface of the membrane to a larger extent than either AChCl or NaCl, its blocking effect becomes predictable.

The membrane potentials induced by the presence of *d*-TCCl gradients are also sensitive to PS concentrations (Fig. 3a; Table 1) and it can be remarked that in the case of *d*-TCCl even in the presence of an amphoteric phospholipid (PC) a clear cationic selectivity was observed.

In a previous work⁸ a difference between choline chloride (ChCl) and AChCl responses was reported when natural extracts were used. This is not the case in this system. Figure 3b and Table 1 indicate that the behaviour in the presence of ChCl is not distinguishable from the behaviour when AChCl is tested.

It has been reported that the membrane potential developed when a NaCl gradient is established across undoped membranes is a function of the net charge of the lipids⁹ used. We have shown that a low percentage of PS is sufficient to develop cationic selectivity (Figure 3c), although, in practice, pure PC membranes did not discriminate between cations and anions.

The present results show that pure lipid membranes containing a small amount of negative lipids can show cationic

selectivity. Furthermore, when the salt gradients are established with cholinergic agents, membrane potentials occur which also show cationic selectivity. The cationic selectivity could be due to a higher permeability for these organic cations when negative lipids are present. Adsorption potentials probably arise, however, at the membrane interface and, consequently, it is not possible to know whether or not a local equilibrium exists at the interface. If the calculated potentials are equal to a distribution potential, or an adsorption potential cannot be established in our experimental conditions¹⁰, these potentials would be similar to those described by Bean *et al.*¹¹ when procaine or other organic molecule gradients were applied across membranes containing brain lipids (whose proportion of negative lipids was not reported).

These types of interactions could be the cause, at least in some cases, of the "cholinergic responses" obtained with membranes containing natural extracts. This is especially true in the case of *d*-TCCl, generally used at concentrations similar to those used here^{2,4,5}. It has been reported that proteolipids could have an important role in cholinergic systems¹² and it is also known that these molecules are rich in negative lipids. When AChCl is applied on artificial membranes containing proteolipids from electric eel a "conductance change" appears, which can be abolished by *d*-TCCl⁸. In view of our results the role of membrane potentials due to the presence of negative lipids must be taken into account when interpreting these type of experiments. Similarly, the *d*-TCCl-dependent membrane potential observed when salt gradients are established across proteolipid containing membranes⁷ could be also dependent on the presence of negative lipids. We can speculate that proteolipids induce negatively charged clusters in the membrane that favour the adsorption of different cations. The role of the interactions described here in the pre- or post-synaptic systems is a matter of discussion.

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potentially harmful agents and it is likely that its detoxifying properties are important.

Gut cells are capable of oxidative, reductive, hydrolytic and various synthetic reactions, including acetylation and conjugation with sulphate and glucuronic acid¹. There are relatively few quantitative data on drug metabolism within the gut mucosa but Conolly *et al.*² demonstrated that the β agonist isoprenaline, administered orally, is excreted principally in the urine as an ethereal sulphate, and their experiments suggested that conjugation took place within the gut wall. Intravenous isoprenaline was excreted in the urine largely unchanged. Sulphate conjugation greatly reduces the systemic availability of isoprenaline which can produce pharmacological effects in μ g quantities intravenously³, but which is used in mg amounts as an oral drug⁴. George, *et al.*⁵ showed that when isoprenaline is placed in an isolated dog gut loop *in situ*, only a small fraction appears as free isoprenaline in the venous effluent draining the loop, the major proportion being a sulphate conjugate. They also demonstrated that inclusion of salicylamide with isoprenaline in the loop resulted in greatly increased concentrations of free isoprenaline in the venous effluent. Salicylamide is also metabolised by sulphate conjugation⁶, and their findings suggested that competition for available sulphate in gut wall enabled more free isoprenaline to drain from the gut. We have investigated this possibility in the intact animal.

Seven female greyhound dogs (20–34 kg) were placed in a restraining stand and remained conscious throughout the experiment. Heart rate was recorded by electrocardiograph. On a control day, after heart rate had stabilised, each dog was given isoprenaline, 3.0 mg base in 200 ml of water by stomach tube. Four dogs also received ³H-isoprenaline (+Isoprenaline-(³H)-HCl, 1.8 Ci mg⁻¹, Radiochemical Centre, Amersham) and their plasma isoprenaline was measured² in systemic venous blood. Heart rate was recorded for 180 min. On a test day, after approximately 10 d, the experiment was repeated except that 600 mg of salicylamide was given with isoprenaline. Five of the dogs also received 600 mg of salicylamide in gelatin capsules by mouth 2 h and 1 h before isoprenaline and salicylamide. Three of the dogs were later given 600 mg of salicylamide and 1.0 g of L-cysteine by mouth 2 h and 1 h before receiving a solution containing 1.0 g of L-cysteine, isoprenaline and salicylamide, by stomach tube.

When isoprenaline alone was given, the mean peak plasma isoprenaline concentration was 2.02 ± 0.28 (s.e.m.) ng ml⁻¹ and the area under the plasma isoprenaline-time curve was 211.7 ± 32.2 ng ml⁻¹ min. After isoprenaline had been given with salicylamide, peak plasma concentration

Table 1 Peak heart rate responses (beats min⁻¹) and areas under heart rate-time curves (beat min⁻¹) in seven conscious dogs given isoprenaline (3.0 mg base), salicylamide (600 mg) or L-cysteine (1.0 g) by mouth as indicated

Dog		Isoprenaline alone	Isoprenaline + salicylamide	Isoprenaline + salicylamide + L-cysteine
A	Peak	56	86	
	Area	2,355	4,889	
B	Peak	52	134	
	Area	2,143	6,082	
C	Peak	70	108	
	Area	2,529	4,719	
D	Peak	45	125	
	Area	1,430	4,435	
E	Peak	74	106	84
	Area	1,468	4,220	2,668
F	Peak	71	151	52
	Area	1,736	3,678	1,888
G	Peak	92	142	111
	Area	3,182	4,420	2,982
Mean	Peak	65.7	121.7	82.3
	Area	2,120.4	4,634.7	2,512.6

Competition for sulphate during detoxification in the gut wall

ALTHOUGH the absorptive function of intestinal cells has been studied in depth, the capacity of intestinal mucosa to metabolise ingested substances has received less attention. But the gut wall presents a first line of defence against

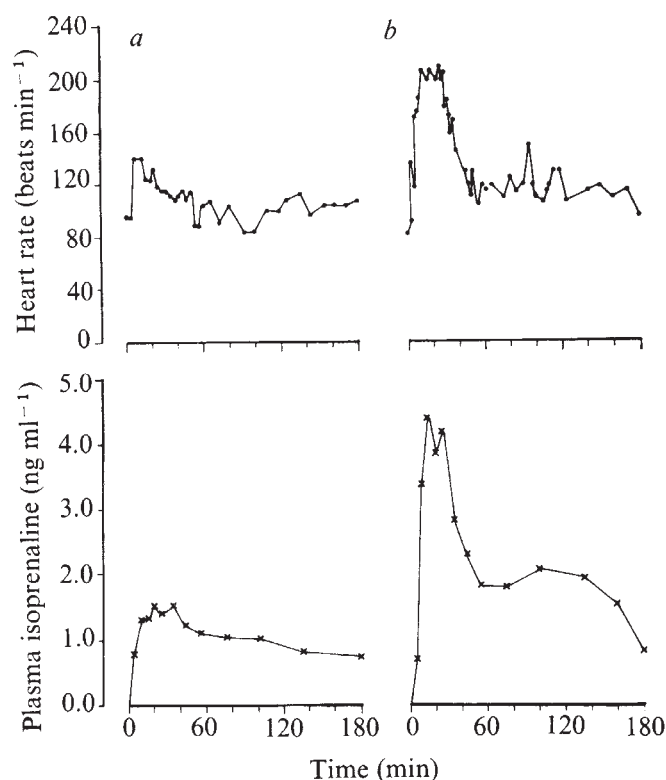


Fig. 1 Plasma free isoprenaline and heart rate responses in a conscious greyhound which received: a, isoprenaline (3.0 mg base) or b, isoprenaline (3.0 mg base) with salicylamide (600 mg) on separate days.

was greater in each animal (mean $4.67 \pm 1.38 \text{ ng ml}^{-1}$) as was the area under the plasma isoprenaline-time curve (mean $386.9 \pm 50.6 \text{ ng ml}^{-1} \text{ min}$). The results of a typical experiment are shown in Fig. 1.

In each animal the heart rate response, expressed as the peak heart rate from baseline and as the area under the heart rate-time curve calculated by trapezoidal rule, was greater when isoprenaline was given with salicylamide than when it was given alone (Table 1). In three animals given L-cysteine with isoprenaline and salicylamide, heart rate responses were less than those for isoprenaline and salicylamide and were similar to the responses for isoprenaline alone.

To assess whether salicylamide has any direct effect on the responsiveness of the dog heart to isoprenaline, an intravenous infusion of isoprenaline sulphate, $0.019 \mu\text{g}$ (base) $\text{kg}^{-1} \text{ min}^{-1}$, was given to a conscious dog which had received atropine, $40 \mu\text{g kg}^{-1}$, intravenously. When a stable increase in heart rate had been achieved, 80 mg of salicylamide was given intravenously. The mean ($\pm$ s.d.) heart rate was $134 \pm 14 \text{ beats min}^{-1}$ for 12.5 min before salicylamide and $130 \pm 12 \text{ beats min}^{-1}$ for 12.5 min after salicylamide. These results are not significantly different at the 5% level of probability.

Sulphate exists in an activated form in the gut⁷ and under the influence of sulphokinases, which exist throughout the gut⁸, it can react with many substances, especially those with phenolic groupings. Salicylamide is metabolised principally to sulphate and glucuronide conjugates, and Levy and Matsuzawa⁶ showed in man that the fraction of the dose excreted as salicylamide sulphate decreased markedly as the oral dose of salicylamide was increased from 75 to 2,000 mg. They also showed that co-administration of L-cysteine, which is catabolised to inorganic sulphate, restored the conjugation of salicylamide even at high doses, indicating that the capacity was limited by the availability

of sulphate. It seems likely that the explanation for the increased plasma isoprenaline and increased cardiac responses when isoprenaline and salicylamide were administered together lies in competition between the two compounds for available sulphate in gut mucosa, enabling more free isoprenaline to reach the systemic circulation. The reduced cardiac response when L-cysteine was administered with salicylamide and isoprenaline is consistent with this hypothesis since availability of sulphate would not in this case be rate limiting. Thus the systemic availability of oral isoprenaline which is normally limited by metabolism in the gut wall can be increased by co-administration of another drug which shares its route of metabolism. Moreover, we have found that the increased availability and pharmacological effects are of considerable magnitude.

Although isoprenaline is commonly administered by inhalation of an aerosol, most of an inhaled dose is known to be swallowed and absorbed through the gut⁹. We have no evidence of interaction between isoprenaline or similar drugs and salicylamide in clinical use, but events of this type may be difficult to identify. One reason may be self-medication, for salicylamide is a constituent of some proprietary mixtures obtainable without prescription. Indeed it is possible that ascorbic acid (vitamin C) which can reduce the conversion of salicylamide to salicylamide sulphate¹⁰, and paracetamol¹¹ which can compete with salicylamide for sulphate may also reduce the sulphate conjugation of isoprenaline, and both these substances are readily available to the public. Thus interactions between sympathomimetics which form conjugates with sulphate and other drugs which are similarly metabolised, seem to be a realistic possibility and would be potentially dangerous.

It is interesting that no clear explanation has been found for the rise in asthma deaths associated with the use of aerosol bronchodilators¹². The interaction we describe illustrates a mechanism whereby toxic amounts of sympathomimetic drugs, even administered by aerosol, may reach the systemic circulation. Viewed from a different aspect, competition between substances which are metabolised by conversion to sulphate conjugates might be used to enhance the availability and therapeutic usefulness of a drug which is normally inactivated by gut mucosa.

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Caffeine inhibits cell transformation by 4-nitroquinoline-1-oxide

SOMATIC mutation has been considered a likely initiating step in chemical carcinogenesis¹⁻³, chiefly because of the close correlation between the mutagenic and carcinogenic activity of various chemicals⁴⁻⁶. Study of cells from patients with disease predisposing to a high incidence of cancer,

such as xeroderma pigmentosum and Fanconi's anaemia, has suggested that defective DNA repair is correlated with this predisposition to malignant transformation. When cells are transformed by 3-methylcholanthrene⁷, 4-nitroquinoline-1-oxide (4NQO)⁸, or X irradiation⁹⁻¹¹, division is necessary soon after treatment to fix the transformation: if treated cells are unable to grow the transformation is not fixed. These results could be explained if DNA damage induced by a carcinogen is converted into a stable and replicable structural change only by means of DNA replication before the intervention of error-free repair mechanisms. The model of mutagenesis¹²⁻¹⁴ based on evidence obtained with micro-organisms suggests that errors introduced by an error-prone postreplication repair mechanism are the principal source of mutation. Furthermore, caffeine inhibits "postreplication repair" (presumably an error-prone mechanism) but not excision repair (presumably error-free) in rodent cells¹⁵⁻¹⁹. I now report that caffeine reduces the transformation frequency of mouse cells treated with 4NQO. This supports the somatic mutation theory of cell transformation.

Malignant transformation was assayed in A31-714 cells, a subclone isolated from BALB/3T3-A31 clone, and 4NQO which induces transformation in these cells after a short exposure²⁰. Actively growing cells were treated with 4NQO for 30 min in suspension, washed and then plated into 60-mm plastic dishes containing 5 ml of Eagle's minimal essential medium supplemented with 10% newborn or foetal bovine serum. Plating efficiency was determined 10 d later,

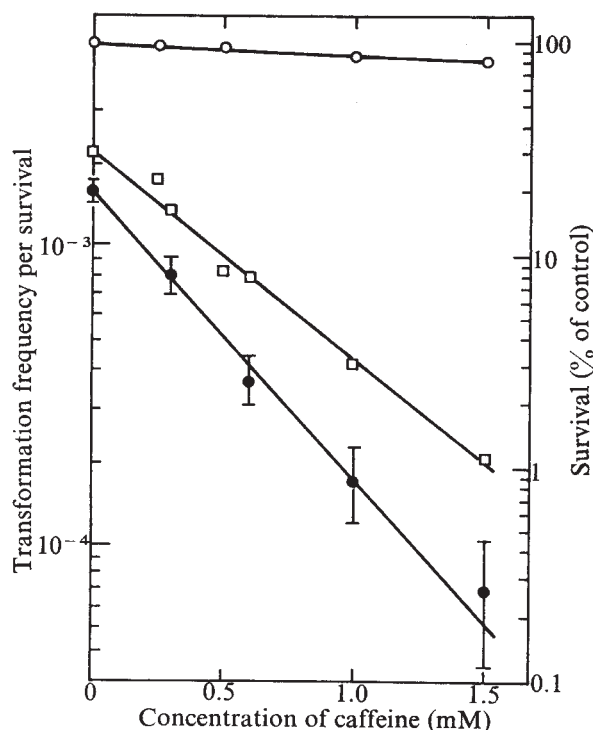


Fig. 1 Survival and transformation frequency in A31-714 mouse cells treated with 4NQO and subsequently incubated with various concentrations of caffeine. The graph shows surviving fraction of untreated ($\circ$), and of 4NQO-treated cells ($\square$); transformation frequency (mean $\pm$ s.e.) of 4NQO-treated cells ($\bullet$). Actively growing cells were treated with 4NQO ($0.1 \mu\text{g ml}^{-1}$) for 30 min in suspension, washed with Dulbecco's PBS, and plated at an inoculum such that about 1×10^4 cells per 5-cm dish survived for the transformation assay and 30–100 cells per dish survived for the assay of plating efficiency. Caffeine was present in the culture medium for the first 48 h after plating. The medium was then replaced with fresh, caffeine-free medium and was subsequently changed twice a week. Dishes were then fixed with methanol and stained with Giemsa either 10 or 30 d after plating and scored for plating efficiency and transformation frequency, respectively. The s.e. of the plating efficiency was always less than 3% of the corresponding mean value.

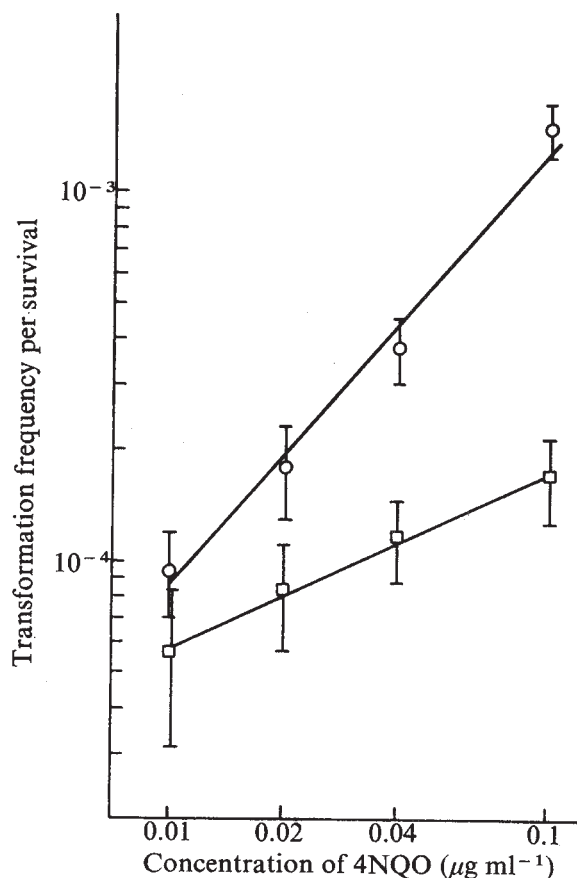


Fig. 2 Transformation frequency (mean $\pm$ s.e.) in A31-714 mouse cells treated with various concentrations of 4NQO in the presence ($\square$) or absence ($\circ$) of 1 mM caffeine for 48 h after treatment with 4NQO. See legend to Fig. 1.

and transformation was scored 30 d after treatment as described previously²⁰. Caffeine was dissolved in Dulbecco's PBS, filtered, and added to culture medium. Because of the need for cell proliferation for the fixation and expression of transformation⁸, plating efficiency was determined initially for all experimental conditions, and then the number of cells plated was adjusted, so that the average number of divisions required to reach confluence for the surviving cells was nearly the same in all dishes.

Figure 1 shows the dose-response curves for both transformation and survival of A31-714 cells treated with 4NQO ($0.1 \mu\text{g ml}^{-1}$) in the presence or absence of caffeine for the first 48 h after treatment. The upper two curves, which represent cell survival, show that caffeine reduced the survival of treated cells but not that of untreated cells. Transformation frequency per surviving cell, shown by the lower curve with the vertical bars, was also reduced by caffeine. Both survival and transformation frequency of treated cells were inversely proportional to the concentration of caffeine and almost parallel within the range of concentrations of caffeine tested. As Fig. 2 shows, the inhibitory effect of caffeine on transformation frequency was also dependent on the concentration of 4NQO.

The exposure of caffeine to cells before, or 48 h after treatment with 4NQO ($0.1 \mu\text{g ml}^{-1}$ for 30 min) had no marked effect on survival and transformation frequency (Table 1). The presence of caffeine for the first 24 h after treatment with 4NQO caused much more reduction in survival and transformation frequency than in successive 24 h periods (Table 1). When the transformation frequency was calculated on the basis of the number of cells inoculated, the inhibitory effect of caffeine was even greater (not shown).

In the conditions of my experiment, most cells which

did not grow rapidly became detached from the substrate, so that 24 h after plating less than 10^5 cells per dish were attached to the substrate, and less than 2×10^4 cells per dish were attached after 48 h. As reported previously⁴, these cell densities affect neither the fixation nor the expression of transformed state.

My results are apparently consistent with the finding that caffeine inhibits tumour induction by ultraviolet irradiation in mice *in vivo*²¹. The discrepancy between my results and those of Donovan and DiPaolo²², in which caffeine appeared to enhance the chemically induced morphological transformation of hamster embryonic cells, may be due to differences between the transformation assays used, or to the length of time and the sequence of carcinogen and caffeine treatment. In their experiments, cells were exposed to carcinogens for 48–49 h simultaneously with, or beginning 1 h before, addition of caffeine.

Table 1 Survival and transformation frequency of 4NQO-treated A31-714 cells in the presence of caffeine added at various times

Presence of 1 mM caffeine (h after 4NQO-treatment)	Plating efficiency 10 d after treatment (%)	Transformation frequency per survival* ($\times 10^{-4}$)
—24–0	26	12.7 ± 1.7
0–48	23	13.9 ± 1.7
0–96	2.4	1.5 ± 0.4
48–96	1.9	1.8 ± 0.6
0–24	16	12.5 ± 2.3
0–48	4.1	3.0 ± 0.8
24–48	11	6.5 ± 1.1

*Mean $\pm$ s.e.

In mammalian cells, the most marked effect of caffeine is its potentiation of cell killing and induction of chromosome aberrations by ultraviolet light and alkylating agents²³. These are caused by low doses of caffeine, which by themselves do not have significant effects on cell survival, as my experiments have also shown. It has been suggested that this synergistic effect of caffeine is due to its inhibition of "postreplication repair", because all results reported so far show that caffeine inhibits the elongation of newly synthesised daughter strand DNA after ultraviolet irradiation of rodent cells^{15–19}. With the A31-714 cells used in this experiment, 1 mM caffeine does not inhibit 4NQO-induced unscheduled DNA synthesis²⁴. I suggest that the reduction by caffeine of survival and transformation frequency of 4NQO-treated A31-714 cells is related to the observation that caffeine inhibits "postreplication repair" in mouse cells. Thus if "postreplication repair" is a viable error-prone repair mechanism, its inhibition would be expected to have the results reported here. This is compatible with the suggestions that (1) cell division soon after carcinogen treatment is required for the fixation of transformation^{7–11}, and (2) late G cells show the highest frequency of transformation by radiomimetic alkylating agent²⁵.

My results seem to support the repair-error hypothesis^{14,24,26} as a mechanism for chemical and physical carcinogenesis. According to this hypothesis, carcinogen-induced DNA damage can be repaired by at least two repair mechanisms, one sensitive to caffeine and the other not. The former may be equivalent to the so-called "postreplication repair" which occurs chiefly during or soon after DNA replication and is presumed to be error prone. The latter, however, may be equivalent to excision repair which occurs throughout the cell cycle, and is presumed to be error free. The errors in the former repair system would then be the main source of mutations, some of which may lead to cell transformation.

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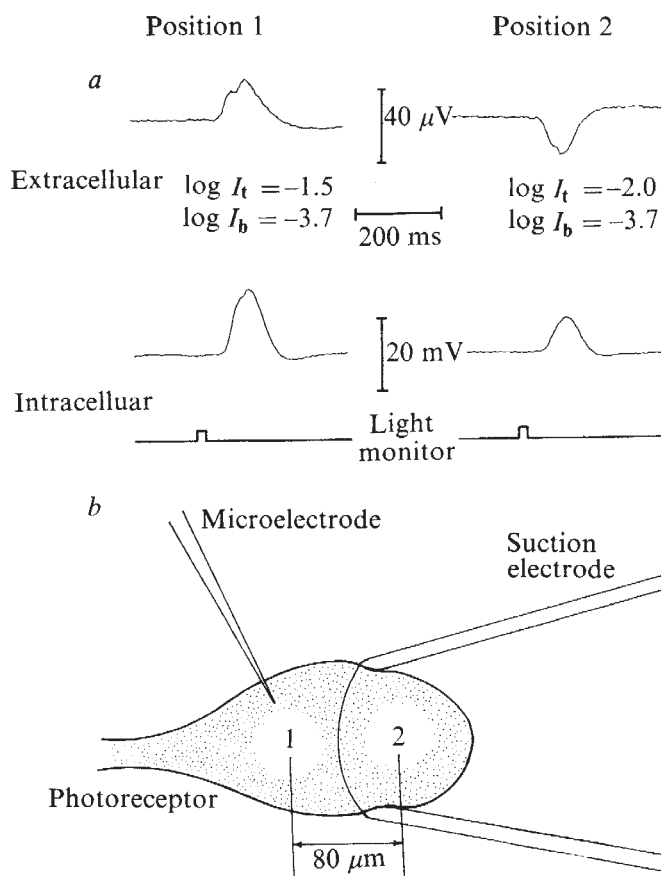
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Local membrane current in *Limulus* photoreceptors

It has been shown¹ that when part of a cell of the ventral photoreceptors of *Limulus* is illuminated, light adaptation is localised to the region of illumination. Since, in the vertebrate retina, there is a well known component of adaptation that is correlated with pigment concentration^{2–4}, local adaptation in *Limulus* may similarly be related to pigment bleaching in the illuminated region of the photoreceptor. This has been shown to be improbable, however, by experiments which indicate that adaptation in the photoreceptor is unrelated to the recovery of the visual pigment^{5,6}.

Another possibility is that local adaptation is related to some process that is initiated by the visual pigment. Voltage clamp studies on ventral photoreceptors⁷ have shown that illumination initiates an inward flow of membrane current, brought about by an increase in the underlying conductance. If the inward current and the underlying conductance increase were localised to the region of illumination, then it might be possible that either, or both, of these processes are in some way responsible for local adaptation. We have therefore attempted to demonstrate directly the flow of local membrane current in the ventral photoreceptors of *Limulus*.

The ventral photoreceptors are located on the so-called lateral 'olfactory' nerve, which was removed from the animal, desheathed, and pinned to the bottom of a chamber with a transparent base. A photoreceptor was stimulated from below, using an optical bench so that a small movable spot of light (nominally 30 μ m diameter in these experiments) could be positioned anywhere on these large photoreceptors (typically $50 \times 150 \mu$ m in cross section⁸). Under visual control ($\times 400$ compound microscope), a portion of a single photoreceptor was gently drawn into a glass suction electrode. Then a KCl filled micropipette (resistance, 10–30 Ω measured in the Ringer that bathed the preparation) was inserted into the cell. We were thus able to measure, simultaneously, the extracellular currents using the suction electrode, and the receptor transmembrane potential using the intracellular pipette. Figure 1b shows a



schematic representation of the recording and stimulating situation. The test spot was located at either of two positions, designated 1 and 2. The aim was to stimulate two different regions of the photoreceptor, one inside, the other outside the suction electrode.

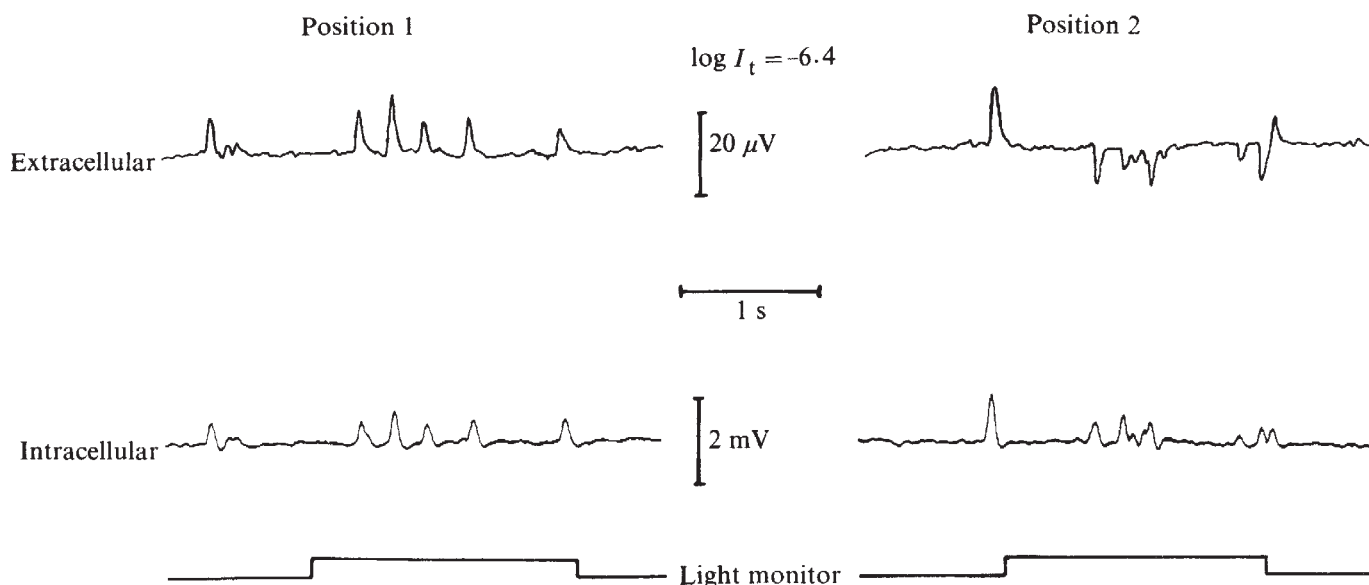
Figure 1 indicates that local illumination gives rise to a localised flow of inward membrane current. When the region inside the suction electrode was illuminated (position 2 Fig. 1), a flow of current from the bath into the suction electrode was measured. The path was completed when the current flowed into the illuminated region of the cell, inside the suction electrode, and out of the cell through the unilluminated regions, outside the suction electrode. Similarly, when the photoreceptor was stimulated at a

Fig. 1a, Local membrane currents produced by local illumination in a light-adapted photoreceptor. b, Schematised version of the recording situation, showing the two stimulus spots labelled 1 and 2. Light intensities (I) are given as $\log_{10} I/I_0$ where I_0 is the intensity of the unattenuated beam. I_t is the intensity of the test flash, and I_b is the intensity of the full-field steady background. The extracellular recording was made between a chloride-coated silver wire inside the suction electrode and a chloride-coated silver wire in the bath. An a.c. coupled amplifier with a 0.1-Hz low frequency cutoff was used for the extracellular recording.

region outside the suction electrode (position 1, Fig. 1b) we measured a flow of current from the suction electrode into the bath. As before, the path was completed when the current flowed into the illuminated region of the cell, this time in the region outside the suction electrode, and out of the cell through the unilluminated regions, this time located inside the suction electrode. In both cases the receptor current depolarised the cell, which was shown by the intracellular recordings of the receptor transmembrane potential. These results indicate that localised illumination of these photoreceptors leads to a flow of local membrane current. This finding is similar to the observation of local membrane currents in the outer segment of squid photoreceptors⁹. The observations on squid photoreceptors, however, did not indicate which changes might bring about the local membrane current. As mentioned previously, the voltage clamp technique has shown that illumination initiates a flow of inward membrane current in *Limulus* ventral photoreceptors, that is brought about by an increase in conductance. Our results therefore indicate that a localised conductance increase gives rise to the local inward membrane current, produced by the localised illumination.

When dark-adapted *Limulus* photoreceptors are stimulated with dim flashes, discrete waves of depolarisation (Fig. 2), sometimes referred to as 'quantum bumps', are observed. These 'quantum bumps' have been observed in a variety of arthropod photoreceptors^{7,10-13}, and in ventral photoreceptors they seem

Fig. 2 Local membrane currents associated with 'quantum bumps' in a dark-adapted photoreceptor. Positions 1 and 2 are the same as those shown in Fig. 1b. The recording situation was the same as that shown in Fig. 1, but the data shown are from a different cell than in Fig. 1. I_t is the intensity of the test flash. Note what seems to be 'spontaneous quantum bumps' preceding the light stimuli. The above records show only one polarity of these 'spontaneous quantum bumps'. In other records, not presented here, we have also observed 'spontaneous quantum bumps' of opposite polarity. These 'quantum bumps' seem to be spontaneous but we cannot rule out the possibility that they are produced by stray light.



to be produced when a photon excites a single rhodopsin molecule¹⁴. It has been proposed¹⁵ that the receptor potential is the summation of these quantum bumps. Figure 2 shows that the individual quantum bumps are associated with local membrane currents. The results presented in Fig. 2 indicate that when a single photon excites one rhodopsin molecule, this process initiates a localised conductance increase which, in turn, generates a flow of local membrane current. We suggest that either the localised light-induced conductance increase, or the local membrane current which it generates, or possibly both, are somehow involved in the local adaptation that follows local illumination.

These results can also be interpreted in conjunction with the Ca^{2+} hypothesis of Lisman and Brown¹⁶. They suggested that a light-induced increase in intracellular Ca^{2+} concentration (Ca_i^{2+}) is a factor controlling light adaptation in *Limulus* ventral photoreceptors. It would seem that following local illumination, the light induced conductance increase and/or the inward current that it initiates, leads to a local rise in Ca_i^{2+} and thereby to local adaptation. This interpretation is supported by the findings of Fein and Lisman¹⁷, who showed that injection of calcium ions into ventral photoreceptors locally desensitised the photoreceptor.

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Intracellular Ca modulates sensitivity and time scale in *Limulus* ventral photoreceptors

IN a photoreceptor cell, light induces a change in membrane voltage (the 'receptor potential'). The sensitivity of the photoreceptor (defined as the amplitude of the receptor response per unit of stimulus energy) depends on its history of illumination. After a cell has been stimulated by light, its sensitivity becomes reduced (that is, the cell becomes 'light adapted'). Fuortes and Hodgkin¹ reported that previous illuminations not only reduced the sensitivity of photoreceptors in *Limulus* lateral eye but also decreased the latency of the responses to subsequent stimuli. They found that the reduction in latency (referred to as a shortened 'time scale') was greater with brighter adapting lights, and that the shortening of the time scale was much smaller proportionally than was the reduction in sensitivity. Similar changes in time scale also have been found in *Limulus* ventral photoreceptors^{2,3}.

It has been proposed that a light-induced increase of intracellular free calcium concentration, Ca_i , normally modulates the sensitivity of *Limulus* ventral photoreceptors⁴. The evidence for this proposal is: (1) intracellular injections of calcium ions decrease the amplitude

of the receptor potentials⁴; (2) intracellular injection of a calcium buffer tends to prevent light-induced changes in sensitivity⁵; and (3) a light-induced rise of Ca_i has been detected directly with the photoprotein aequorin⁶. Here we show that iontophoretic injection of Ca into *Limulus* ventral photoreceptors not only decreases the sensitivity but also shortens the time scale.

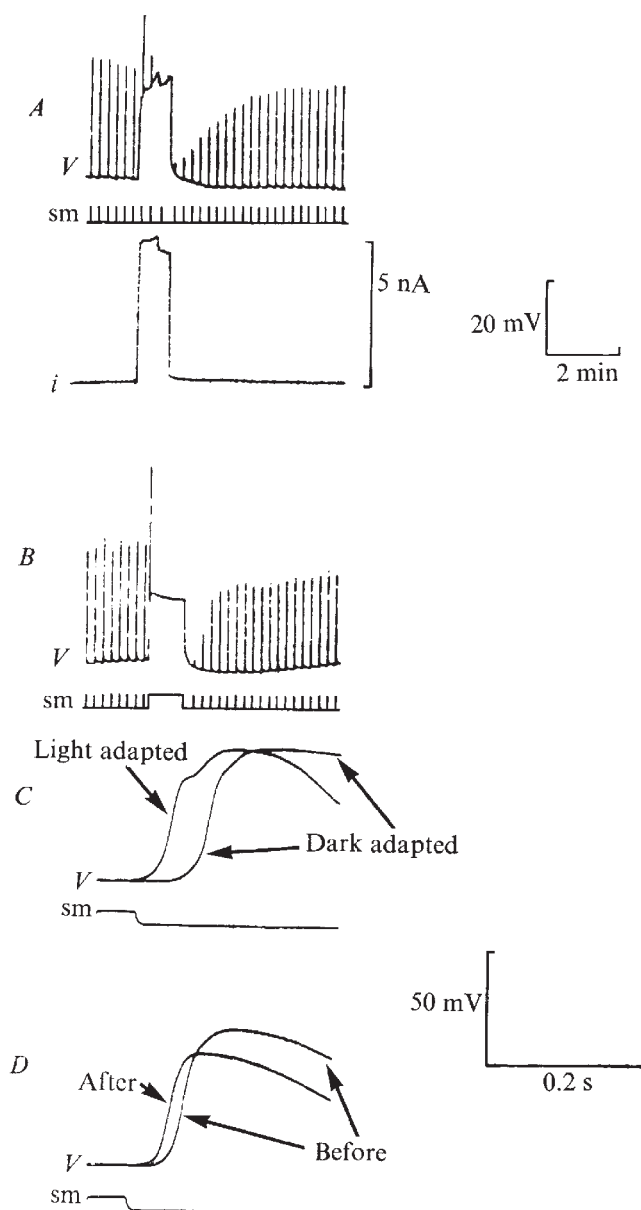


Fig. 1 The amplitude and latency of receptor potentials recorded from *Limulus* ventral photoreceptors, altered by bright lights or by intracellular injection of calcium. *V*, Voltage; *sm*, monitor of the time of occurrence of stimulus light. A micropipette filled with 3 M KCl was used to measure membrane voltage; a second micropipette filled with 0.09 M CaOH , 0.1 M EGTA and 0.1 M Tris base was used to pass Ca iontophoretically into the cell (refs 4 and 11). Unattenuated stimulus beam was $5 \times 10^{-3} \text{ W cm}^{-2}$ on the preparation. *A*, Responses to repetitive brief (20 ms) test flashes (stimulus beam attenuated by 3.9 neutral density) before and after the iontophoretic injection of Ca_i monitor of the current through the electrode containing Ca. *B*, Responses to repetitive brief (20 ms) test flashes (attenuation = 4.8 neutral density) before and after a prolonged adapting stimulus. After the adapting light, the responses to test flashes gradually recovered. Same cell as *A*. *C*, In another cell, the response to a flash (attenuation = 3.6 neutral density) before (dark adapted for 15 min) and 10 s after an unattenuated adapting stimulus. *D*, Same cell as *C*. The response to a flash (attenuation = 3.6 neutral density) before and 10 s after iontophoretic injection of Ca. The cell had been dark adapted for 15 min before each flash.

As described previously⁴, receptor potentials elicited by brief repetitive flashes are attenuated during the iontophoretic injection of Ca, and recover gradually after the injection (Fig. 1A). This phenomenon qualitatively mimics the desensitisation of the cell by an adapting light and the subsequent recovery of sensitivity (Fig. 1B).

It has been inferred⁴ that the attenuation of the receptor potential by intracellular injection of calcium ions is probably due to the attenuation of the light-induced conductance. To examine directly the effects of injection of calcium ions on the light-induced conductance, cells were voltage clamped during the injection. A cell was impaled with two electrodes filled with KCl, for the voltage clamp, and also impaled with a third electrode for injecting calcium ions iontophoretically. The voltage clamp supplied a current equal and opposite to the current passed out of the Ca-containing electrode; if the inside of the cell was equipotential, no net current would cross the cell membrane due to Ca iontophoresis. Before a cell was voltage clamped, the voltages (receptor potentials and resting voltage) recorded by all three electrodes were found to be nearly identical (Fig. 2A). The cell was then voltage clamped, and the currents induced by brief dim repetitive flashes were measured. During the iontophoretic injection of Ca the light-induced current became progressively smaller, and after the injection it progressively recovered (Fig. 2B). The intensity of the repetitive flashes was chosen to be within

the linear portion of the relationship between flash intensity and peak light-induced current; therefore, the reduction in light-induced current was directly proportional to the reduction in the sensitivity of the cell. Thus, the direct intracellular injection of Ca caused a reduction of the sensitivity of the photoreceptor, in the absence of a change of membrane voltage. We have also found that intracellular iontophoretic injection of calcium ions does not noticeably change the reversal voltage of the current induced by a brief flash. Thus, with membrane voltage held constant, the light-induced current is directly proportional to the light-induced conductance. We conclude that the finding illustrated in Fig. 2B directly demonstrates that an increase of intracellular free calcium modulates the light-induced change in membrane conductance which underlies the photoreceptor response. It has been similarly demonstrated that bright illumination reduces the conductance increase evoked by subsequent stimuli⁷.

Using brief bright stimuli, a dramatic shortening of the time scale of the receptor potential was found after the photoreceptor was light adapted (Fig. 1C). To see if an increase of Ca_i affected the time scale in addition to reducing the sensitivity, we measured membrane voltage during and after iontophoretic injection of calcium ions. Shortening of time scale also occurred after the intracellular iontophoretic injection of Ca (Fig. 1D). Moreover, the shortening of time scale did not depend on passing net current across the cell membrane during the iontophoretic injection; injection of Ca while the cell was voltage clamped shortened the time scale (Fig. 2C) and also decreased the sensitivity. Thus, increasing intracellular free calcium mimics two effects of previous illumination: a large reduction of sensitivity and a proportionally smaller shortening of the time scale. Injection of calcium ions does not, however, mimic the excitatory effect of light. Figure 2 shows that membrane current in the dark (measured at resting voltage) was not affected by the injection. This clearly shows that, in *Limulus*, a rise of Ca_i is not the signal for excitation, as has been proposed for vertebrate photoreceptors^{8,9}.

In summary, we have found that in ventral photoreceptors, changing a single quantity, the free intracellular calcium, leads to both a shortening of time scale and a reduction of sensitivity. Increasing calcium concentration has been found to attenuate stimulus-induced changes of membrane conductance channels in other membranes (compare 'stabilisation' effects of Ca; ref. 10). Our findings that intracellular injection of Ca not only decreases the sensitivity but also shortens time scale of the light-induced current suggests that the action of Ca_i is probably not a nonspecific effect on the conductance channels. These findings further support the hypothesis that a change of Ca_i specifically participates in the physiology of light adaptation in *Limulus* ventral photoreceptors.

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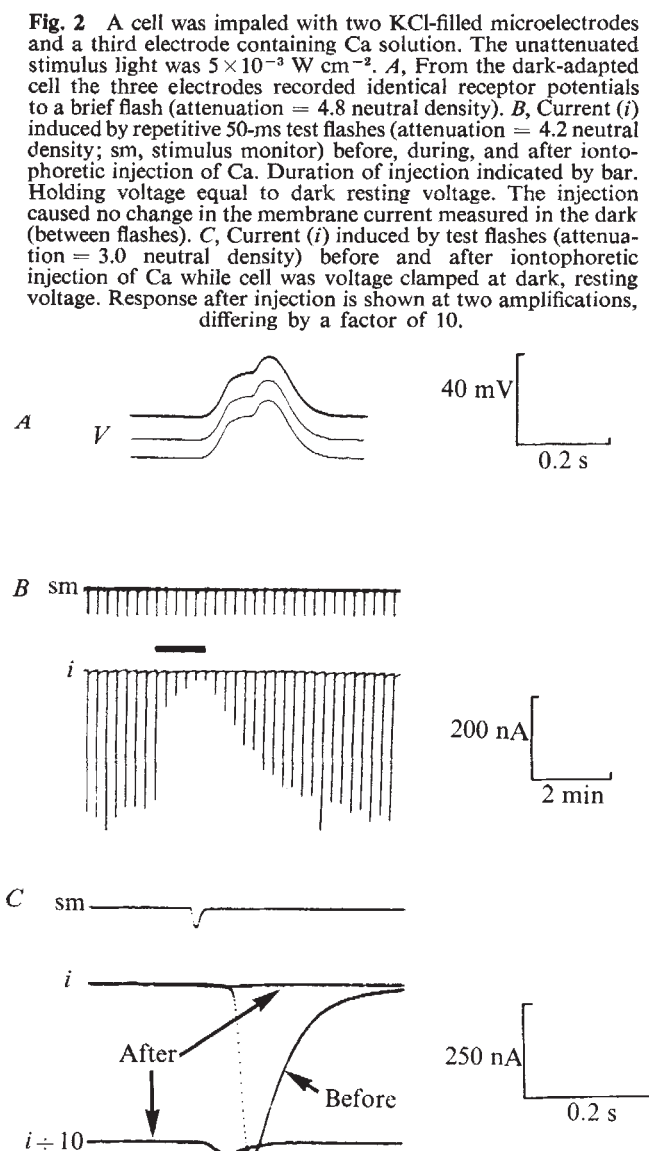
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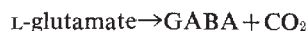
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Selective release of newly synthesised and newly captured GABA from synaptosomes by potassium depolarisation

NORADRENALINE and acetylcholine synthesised *de novo* are released in preference to the endogenous store following nerve depolarisation. Kopin *et al.*¹ showed that the specific activity of noradrenaline (¹⁴C-tyrosine precursor) released from cat spleen during sympathetic nerve stimulation is greater than that of the tissue store; however, ³H-noradrenaline which was just taken up by the nerve is released concomitantly with the endogenous store. In similar experiments, newly synthesised acetylcholine is selectively released from superior cervical ganglia², phrenic nerve³, *Torpedo* electroplax⁴, and brain cortical slices⁵. We used rat cortical synaptosomes (pinched off nerve endings) to study the temporal relationships of GABA biosynthesis, uptake and release.

Suspensions, enriched in synaptosomes, prepared by standard subcellular fractionation techniques, take up alleged neurotransmitter substances (for example, γ -aminobutyric acid (GABA), L-glutamate, L-aspartate) against a concentration gradient by specific sodium-dependent high affinity (low K_m) transport systems⁶. These high affinity transport systems are thought to be important in inactivating neurotransmitter action. Following electrical stimulation⁷ or calcium-dependent potassium depolarisation^{8,9}, preparations enriched in synaptosomes specifically release neurophysiologically active amino acids (GABA, L-glutamate, L-aspartate). Using ¹⁴C-L-glutamate as a precursor to measure GABA biosynthesis *de novo* and low concentrations of ³H-GABA as a substrate for the high affinity uptake system, we find that newly synthesised GABA is released from synaptosomes concomitantly with the GABA taken up by the high affinity transport system. Moreover, both newly synthesised and captured GABA are released in preference to endogenous GABA stores.

GABA is a probable transmitter in the vertebrate central nervous system¹⁰ and the established neurotransmitter at the crustacean neuromuscular junction¹¹. Although usually considered an inhibitory neurotransmitter, we suggested that GABA may be the excitatory transmitter of the olfactory nerve in the gar fish and pike¹². Glutamate decarboxylase catalyses the biosynthesis of GABA according to



This enzyme, which requires pyridoxal phosphate¹³, is strongly inhibited by carbonyl-trapping reagents such as hydroxylamine and hydrazine¹⁴, and also by mercaptosuccinate (our unpublished observations).

Using male Sprague-Dawley rats (200-250 g), fractions enriched in synaptosomes were isolated from crude mitochondrial suspensions on discontinuous Ficoll gradients¹⁵ and stored at 0 °C in calcium-free Ringer⁹ containing 20 mM morpholinopropane sulphonic acid (pH 7.4). All incubations were completed within 7 h after the animals were killed. To remove ¹⁴C-GABA and other impurities, commercial ¹⁴C-(U)-L-glutamate (237 Ci mol⁻¹) was subjected to paper electrophoresis in 8% acetic acid-2% formic acid. Commercial ³H-GABA (36.7 Ci mmol⁻¹) was free from impurities using this system. Preparations enriched in synaptosomes always contain adventitious microsomes and myelin. Control studies which we report show that the conclusions are unaltered by this contamination.

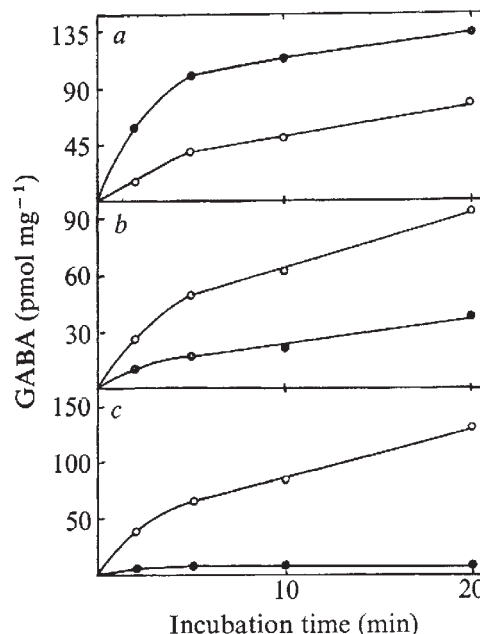


Fig. 1 Variation of newly synthesised and newly captured GABA in synaptosomes by changing exogenous substrate concentrations. A 90- μ l aliquot of synaptosomal suspension (50-60 μ g protein) was incubated at 25 °C for the time indicated with 10 μ l of solution containing ¹⁴C-L-glutamate and ³H-GABA in Ringer's solution to give the following final respective concentrations: a, 30 μ M-0.6 μ M; b, 30 μ M-0.3 μ M; c, 30 μ M-0.05 μ M. Synaptosomes were collected on Millipore filters (0.45- μ m pore). The filters were placed on Whatman 3MM paper and electrophoresed in 8% acetic acid-2% formic acid at 30 V cm⁻¹. After drying, carrier GABA was visualised with ninhydrin and quantitatively analysed for newly synthesised (¹⁴C) (○) and newly captured (³H) (●) GABA in Tritonol¹⁸ using the channel ratios method¹⁹. Results are expressed per mg synaptosomal protein. In the conditions used, tritiated GABA was 17,000 c.p.m. pmol⁻¹ and ¹⁴C-GABA (newly synthesised) was 94 c.p.m. pmol⁻¹. Similar results were obtained in 2-4 independent experiments.

After incubating synaptosome fractions with ¹⁴C-L-glutamate (30 μ M) and ³H-GABA (0.6 μ M) at 25 °C, the rate of accumulation of both radiolabels into synaptosomes isolated by Millipore filtration is linear for 4 min and gradually flattens out between 20 and 30 min. To assess the extent of metabolism of these substances, including the conversion of L-glutamate to GABA, the synaptosomal preparation was collected by centrifugation (6,000g, 5 min, 4 °C) after incubation for 30 min with each labelled precursor. Subsequently, a water extract of the synaptosomes was prepared, applied directly on to paper, and electrophoresed. The position of the label was determined with a radiochromatogram scanner and that of the amino acids was determined by the ninhydrin reaction. About 80-90% of the radiolabel comigrated with the precursor. Moreover, L-glutamate (3%) was converted into a substance which comigrated with GABA during paper electrophoresis using 5.5% acetic acid-pyridine (pH 4.2) or the acetic acid-formic acid system. Incubation of the synaptosomes with hydroxylamine (1 mM) or mercaptosuccinate (1 mM) inhibits GABA bioformation from ¹⁴C-L-glutamate by 90% and 25%, respectively, as determined after resolving sequestered GABA and L-glutamate by paper electrophoresis. In addition the ratio of newly synthesised GABA (¹⁴C-L-glutamate precursor) and newly captured GABA (³H-GABA precursor) varies with the concentration of precursor used in the synaptosomal incubation medium (Fig. 1a-c). Decreasing the concentration of ³H-GABA in the medium, for example, decreases its uptake into synaptosomes with an attendant increase in the ratio of ¹⁴C-GABA (newly synthesised) to ³H-GABA (newly captured).

After documenting the biosynthesis of GABA from

^{14}C -L-glutamate and the uptake of ^3H -GABA into the synaptosome preparation, the effect of potassium depolarisation on the release of newly synthesised and captured GABA was studied. Synaptosomes containing ^{14}C - and ^3H -GABA release the candidate neurotransmitter on treatment with 56 mM potassium–1 mM calcium (Fig. 2a–c). The ratio of newly synthesised to newly captured GABA (labelling ratio) released closely parallels that found intrasynaptosomally before (Fig. 2a–c) and after (data not shown) potassium depolarisation. In the data shown (Fig. 2a–c), the labelling ratios of intrasynaptosomal and released GABA were (a) 0.58; (b) 2.49; (c) 22.8 ($\pm 8\%$). This shows that the GABA released (up to 70%) by potassium depolarisation consists of both newly captured and synthesised substance. Although the intrasynaptosomal ratio of these two forms of GABA can be controlled by varying substrate concentrations, neither form is selectively released. These results reflect net release since the magnitude of re-uptake of labelled GABA during depolarisation cannot be estimated.

Next, the specific radioactivity of stored and released GABA was measured. After depolarisation, the material released initially contains newly synthesised (^{14}C) and captured GABA (^3H) of higher specific activity than that found in the synaptosomes before potassium–calcium treatment (Table 1). With longer incubations, the specific activity of each label decreases to a value similar to that found in the initial preparation. These results support the hypothesis that newly synthesised and captured GABA are released preferentially when compared with the endogenous GABA store.

Since myelin and microsomes sequester GABA⁹, experiments were carried out to test the effect of potassium depolarisation on GABA release from these contaminants. Experiments performed with synaptosome preparations demonstrate that part of the sequestered radiolabel sediments with the myelin–microsomal fraction on sucrose density gradients, whereas the major portion comigrates with the synaptosomal fraction¹⁶. Depolarisation of the preparation enriched in synaptosomes substantially reduces label comigrating with the synaptosomal fractions, but fails to release label from the myelin–microsomal fractions. In a control experiment using a myelin–microsomal preparation, depolarisation also fails to release label. These results are consistent with the observations of Levy *et al.*, who demonstrated that myelin and microsomal preparations fail to release GABA by potassium treatment⁹. Our experiments demonstrate that release of labelled GABA from synaptosome preparations by potassium depolarisation is not adventitious, and confirms the contention that stimulus-induced release is synaptosome specific.

Table 1 Release of endogenous, newly synthesised and newly captured GABA by depolarisation

Time (s)	Total GABA released (nmol mg ⁻¹)	Synthesised (pmol per nmol total GABA)	Captured (pmol per nmol total GABA)
2	< 0.25	> 40	> 26
20	0.74	20.1	15.7
80	1.48	14.4	11.8
150	1.78	11.8	9.5

A synaptosome preparation (870 μg protein) was incubated in a final volume of 300 μl of Ringer containing 30 μM ^{14}C -L-glutamate and 0.6 μM ^3H -GABA for 20 min at 25°C. Synaptosomes were collected, washed and depolarised for the time indicated with K^+ – Ca^{2+} as described in Fig. 2. Fractions containing released GABA were lyophilised and redissolved in 250 μl of 10 mM acetic acid. Total GABA was measured by the enzymatic fluorimetric procedure of Kravitz and Potter²⁰. Newly synthesised (^{14}C) and newly captured (^3H) GABA were determined as described in Fig. 2. Intrasynaptosomal levels of GABA (before depolarisation) were: total GABA—8.7 nmol mg⁻¹ protein; newly synthesised—12.3 pmol per nmol total GABA; newly captured—8.3 pmol per nmol total GABA. All determinations were performed in triplicate. Average values (range $\pm 9\%$) are given.

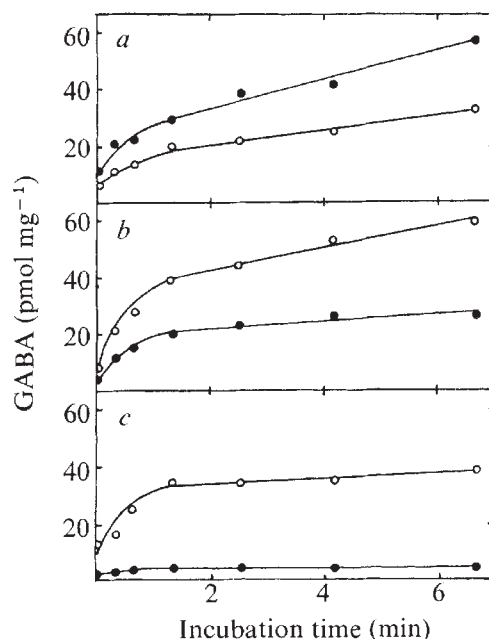


Fig. 2 Release of newly synthesised and newly captured GABA from synaptosomes. Synaptosomes were labelled with ^{14}C - and ^3H -precursor as described in Fig. 1a–c for 20 min, using 120–130 μg of synaptosomal protein in a final incubation volume of 250 μl . Synaptosomes were collected by centrifugation at 4,500g for 3 min and washed twice with 2.5 ml of cold Ringer to remove extrasynaptosomal isotope. The pellet was resuspended in 250 μl of Ringer (on ice); then 250 μl of Ringer containing 112 mM potassium chloride and 2 mM calcium chloride was added rapidly. After incubation at 25°C for the time indicated, the components released into the medium were resolved from synaptosomes by Millipore filtration. The filtrates were lyophilised and redissolved in 100 mM acetic acid containing carrier GABA. Aliquots were electrophoresed on Whatman 3MM paper for 90 min in 5.5% acetic acid–pyridine, pH 4.2 at 15 V cm⁻¹. After drying, GABA was quantitatively analysed as described in Fig. 1. In the conditions used, the following concentrations (pmol mg⁻¹) of newly synthesised and newly captured GABA (respectively) were present before potassium depolarisation (see Fig. 1a–c): a, 80–137; b, 97–39; c, 130–5.7. ○, Newly synthesised; ●, newly captured. Similar results were obtained in 2–4 independent experiments.

A 30- μM concentration of L-glutamate is an effective precursor of GABA in the synaptosome preparation. The K_m of the glutamate high affinity uptake system, the process presumed to inactivate the action of this excitatory neurotransmitter candidate, is about 20 μM (ref. 17). Whether glutamate transport into neurones using GABA as the neurotransmitter is mediated by a similar high affinity transport system remains to be clarified. Newly captured and newly synthesised GABA are released in ratios similar to those found in synaptosomes before depolarisation. Release of GABA is thus unlike that of noradrenaline in the peripheral sympathetic nervous system (splenic nerve) of cat, where the newly synthesised transmitter is released in preference to that which is newly captured. Based on our experiments with rat cortical synaptosomes, GABA reuptake is not only important for terminating transmitter action, but seems to serve as a rather direct source for release of GABA in subsequent nerve volleys.

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Purified hormones from the crustacean eyestalk and their physiological specificity

THE amino acid compositions of two peptide hormones, both acting on the pigmentary-effector system of crustaceans, have been reported¹. One of these, erythrophore concentrating hormone (ECH) has a major function in the rapid physiological colour changes of crustaceans, by concentrating pigment granules of the erythrophores, and is the first crustacean eyestalk hormone described². The second eyestalk hormone, discovered not long afterwards³, moves the distal (DRP) and reflecting retinal pigments of dark-adapted *Palaemonetes* and *Palaemon* into the light-adapted position, but purified hormone has so far been tested on only the DRP. A third well characterised hormone, hyperglycaemic hormone (HGH), increases glucose concentration of the blood, but such hyperglycaemic responses may vary in cross tests among crustacean species^{4–6}. The amino acid composition of HGH has been determined. A summary of six analyses of five different preparations of HGH from eyestalks of *Cancer magister* is shown in Table 1; specific analyses for tryptophan have not been made. Details of the separation and purification will be reported elsewhere.

The diversity of physiological responses produced by hormones from the crustacean eyestalk^{7–9} raises the question of the number of different hormones involved, that is, is each type of response produced by a single hormone, or may one hormone be responsible for more than one effect?

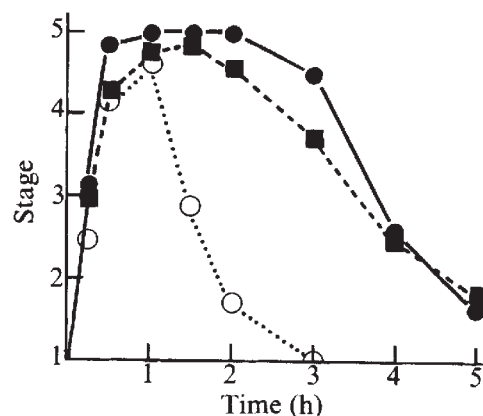


Fig. 1 Effects of synthetic light-adapting DRPH on chromatophores of *U. pugnator*. ●, Melanophores (10 animals); ■, erythrophores (10 animals); ○, leukophores (20 animals). Both eyestalks were removed from male crabs, average wet weight, 3.8 g (range 3.5–4.2 g) 18 h before testing to ensure that, at injection, melanophores and erythrophores were in stage 1. Normal males, averaging 1.6 g wet weight (range 1.4–2.1 g), were adapted to illuminated black backgrounds in individual fingerbowls so that the leukophore pigment was fully concentrated; after injection they were kept in the black containers except during the short intervals for observing the leukophore stage. The large individuals were injected with 0.2 µg and the small ones 0.1 µg of synthetic hormone (105 µg dissolved in 1.0 ml Pantin's saline¹⁵ containing 1 µl of thiodiethanol). The hormone was stored at –20 °C between injections of groups of crabs. Chromatophore stages were read at $\times 36$ – $\times 54$ magnification.

The ideal approach is to test the specificity of response to pure hormone.

ECH has been tested for its physiological specificity on three crustacean species. Doses of 10^{-8} g of synthetic ECH concentrated the erythrophore pigments in *Uca pugnator* and in *Cambarellus shufeldti*, but were without effect on leukophores and melanophores of these species¹⁰. On the other hand, synthetic ECH in doses of 5×10^{-11} g, as well as hormone purified from eyestalks of *Pandalus borealis*, concentrated pigment both in leukophores and erythrophores of *Palaemon adspersus*¹¹ without eyestalks. The maximum leukophore response was small, an average stage 2.5 (on the Hogben and Slome¹² scale of 1–5) being reached 6 min after injection, with pigment fully dispersed again after the next 25 min. Although control injections are not described and leukophore responses to a range of doses of synthetic ECH are not reported, the results indicate differences in specificity of response to this hormone among crustacean species.

Table 1 Amino acid composition of hyperglycaemic hormone (HGH) from eyestalks of *C. magister*

Amino acid	140–1	142–3	144–5	146–7C	146–7D	148–9A	Average number of residues	Range used	Estimated round number of residues
Lysine	2.5	4.0†	2.2	1.9	2.0	2.2	2.16	1.9–2.5	2
Histidine	1.0	1.6	0.9	0.8	1.3	1.2	1.13	0.9–1.6	1
Arginine	2.2†	3.5	3.5	3.3	3.0	3.2	3.30	3.0–3.5	3–4
Aspartic acid	7.7	6.6†	8.0	8.2	7.6	7.8	7.86	7.6–8.2	8
Threonine	2.9	2.3	2.2	2.5	2.7	2.2	2.47	2.2–2.9	2–3
Serine	6.8†	6.8†	5.1	4.5	4.6	4.3	4.62	4.3–5.1	4–5
Glutamic acid	7.4	7.5	7.4	6.9	6.9	6.8	7.15	6.8–7.5	7
Proline	2.2	2.0	1.3†	2.0	2.7	2.3	2.24	2.0–2.7	2
Glycine	10.0†	6.4	4.8	5.6	5.5	4.4	5.34	4.4–6.4	5–6
Alanine	4.8	4.0	4.3	4.3	4.6	4.3	4.38	4.0–4.8	4–5
Half cystine*	0.6†	0.8†	1.4	1.1	1.1	3.8†	1.20	1.1–1.4	1–2
Valine†	3.0†	2.9†	3.9	3.8	3.9	3.9	3.87	3.8–3.9	4
Methionine*	0.6†	0.8†	0.8†	1.8	1.8	1.4	1.67	1.4–1.8	2 or more
Isoleucine*	1.0†	1.3	1.5	1.4	1.6	1.6	1.48	1.3–1.6	2
Leucine	3.7†	3.6†	5.5	5.4	4.5	5.4	5.20	4.5–5.5	5
Tyrosine	3.6	3.0	4.0	3.8	3.7	4.2	3.71	3.0–4.2	4
Phenylalanine	2.0	2.0	2.2	2.2	2.3	2.3	2.17	2.0–2.3	2

*Cysteine, methionine and to some extent isoleucine are always low; also tryptophan if present.

†Valine not increased by 70-h hydrolysis time.

‡Values not used in averaging number of residues.

During purification studies on light-adapting DRPH^{13,14}, successive chromatography of eyestalk extract on columns of Sephadex G-25, DEAE-cellulose, and CM-cellulose revealed molecular polymorphism of the hormone. Each molecular version, in addition to light-adapting DRP, also dispersed pigments in melanophores, erythrophores and leukophores of *U. pugnator*. Such persistent association of physiological responses by the four pigmentary effectors indicated the likelihood of molecular identity between light-adapting DRPH and dispersers of chromatophoral pigments.

This possibility was tested with synthetic light-adapting DRPH on *Uca* (Fig. 1). Pigment became dispersed in the three types of chromatophores, the maximum response occurring 30–60 min after hormone injection. Leukophores seem to be less sensitive to the hormone than melanophores and erythrophores. Doses of 2 ng produced an average stage 3.8 for melanophores in 6 eyestalkless *Uca* by 30 min after injection (not shown); the melanophores had returned to stage 1 after 3 h. Control injections of saline-thiodiethanol were without effect on the three kinds of chromatophores; five control animals were used for each chromatophore type.

The results show that light-adapting DRPH is also a disperser of chromatophoral pigments in *Uca*, and that a single hormone may elicit more than one physiological response. The role of purified HGH in the reported activation of phosphorylase and inhibition of uridine-diphosphate-glucose glycogen transglucosylase by eyestalk hormone^{16,18} will be investigated.

Professor Rainer Keller, Drs Klaus-Dieter Spindler and Margaret Spindler-Barth were coworkers in the purification of HGH from *C. magister*; amino acid analyses of this HGH were done by Dr B. Brimhall (University of Oregon Medical School). Dr Per Fernlund provided a sample of synthetic light-adapting DRPH. Grants from the National Institutes of Health and from the National Science Foundation supported this research.

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Rabbit serum also contains an androgen-binding protein (TeBG) with properties very similar to ABP^{8–11}. In rabbits, ABP and TeBG are indistinguishable by size, stability and steroid-binding properties¹² (Table 1). To compare ABP and TeBG further, we have used antisera raised against purified ABP to investigate whether or not ABP and TeBG show immunological cross reactivity.

ABP was purified from 3,000 epididymides of adult rabbits by ammonium sulphate precipitation, ion exchange chromatography on DEAE-cellulose, gel filtration on Sephadex G-200, hydroxylapatite chromatography and preparative polyacrylamide gel electrophoresis¹³. In the purified preparation there was a thousandfold increase in specific ABP-binding activity compared with that of the 105,000g supernatant. Purified (200 µg) or partially purified ABP (12 mg) was injected into young female guinea pigs after emulsification in 0.5 ml of Freund's complete adjuvant. The animals were bled after 6 weeks and those showing an ABP titre were given booster injections to increase the titre. Antisera were checked for precipitin lines by double diffusion tests in agar¹⁴, and by inhibition of ABP-binding activity as measured by steady-state polyacrylamide gel electrophoresis¹⁵.

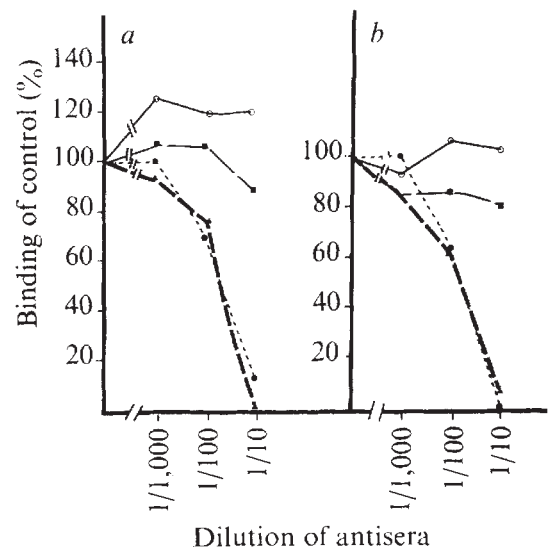


Fig. 1 Inhibition of ³H-DHT binding to ABP (a) and TeBG (b) by different dilutions of anti-ABP antisera. Four guinea pigs were injected with partially purified rabbit ABP in Freund's complete adjuvant, and bled after 6 weeks. Various dilutions of antiserum (1/10, 1/100, 1/1,000) were added to an equal volume of partially purified ABP or immature rabbit serum (diluted 1:50, v/v). Normal guinea pig serum in the same dilution served as controls. After incubation for 48 h at 2–4 °C the samples were centrifuged at 10,000g for 15 min and binding of ³H-DHT to ABP and TeBG was analysed by steady-state polyacrylamide gel electrophoresis¹⁵. There was no visible precipitation at any dilution of antisera. ○, AS1; ●, AS2; ■, AS3; ▲, AS4.

As Fig. 1a shows, antisera from animals 2 and 4 caused 100% inhibition of ABP-binding activity at a dilution of 1:10. Some inhibition was also observed at a dilution of 1:100 but none at 1:1,000. Antisera from animals 1 and 3 showed little or no inhibition of steroid-binding activity, although they had activities against other antigens in the partially purified ABP preparation, as demonstrated by double diffusion tests in agar. Figure 1b shows that antisera raised in animals 2 and 4 also inhibited binding of ³H-DHT to TeBG, and inhibition at different dilutions of antisera was identical to that of the inhibition of ABP-binding activity. The finding that all four antisera showed precipitin lines in double diffusion tests in agar, and only two had a detectable titre against ABP and TeBG suggested that the inhibition was caused by specific antibody to ABP and TeBG and not by an artefact due to coprecipitation with contaminating antigens.

Immunological cross reactivity between testicular androgen-binding protein and serum testosterone-binding globulin

WE have reported the presence of a testicular androgen-binding protein (ABP) which is secreted into the testicular fluid of several mammalian species¹. ABP is produced by the Sertoli cells and is specifically stimulated by follicle stimulating hormone (FSH)^{1–7}. It is found in particularly high concentrations (3×10^{-7} M) in the efferent duct fluid of rabbits.

Table 1 Physicochemical properties of rabbit ABP and TeBG

	ABP	TeBG
$S_{20,w}$	4.7S (12) 4.5S (13)	4.4S (12)
Stokes radius (r_s)	43 Å (12)	44 Å (12)
Molecular weight ($S_{20,w}$, r_s)	74,000 (12)	76,000 (12)
Molecular radius (PAGE)	2.74 nm (12)	2.74 nm (12)
f/f_0 (molecular weight, r_s)	1.61 (12)	1.55 (12)
Molecular weight (PAGE)	75,000 (12)	75,000 (12)
Molecular weight (SDS-PAGE)	68,000 (13)	
Molecular weight (equilibrium ultracentrifugation)	65,500 (13)	
pI	4.7 (12, 13)	5.4 (12)
K_a^{DHT} at 0 °C	$1.6 \times 10^9 \text{ M}^{-1}$ (12)	$1.9 \times 10^9 \text{ M}^{-1}$ (12)
Steroid specificity	DHT > adiol > testosterone > progesterone > androstenedione > cortisol (12)	
Stability		
Binding destroyed by heat	> 50 °C (12)	> 50 °C (12)
Dissociation rate DHT-ABP complex ($t_{1/2}$ at 0 °C)	6 min*	5.2 min (11)

($v = 0.70 \text{ cm}^3 \text{ g}^{-1}$). PAGE, polyacrylamide gel electrophoresis; SDS-PAGE, PAGE in 0.1 % sodium dodecyl sulphate; pI , isoelectric focusing; f/f_0 , frictional ratio. DHT, dihydrotestosterone; adiol, 5 α -androstane-3 α ,17 β -diol. References are given in parentheses.

*Our unpublished results.

One explanation for the apparent immunological similarity between ABP and TeBG could be that TeBG is one of the proteins contaminating the ABP preparations used for immunisation, and that antibodies to both ABP and TeBG had been developed. To examine this possibility, more guinea pigs were immunised and antisera from different animals and bleedings were tested for inhibition of steroid binding to ABP and TeBG. As Fig. 2 shows, all antisera that inhibited steroid binding to ABP also inhibited binding to TeBG. A plot of the binding to ABP against the binding to TeBG gave a positive correlation of 0.985. This high correlation between inhibition of steroid binding to ABP and TeBG strongly indicates that binding to both proteins is inhibited by the same antibody, and not by the products of different immune responses.

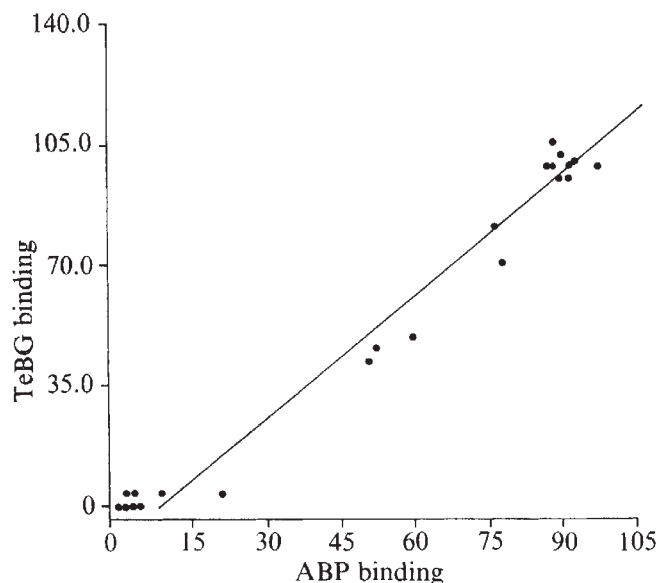


Fig. 2 Effect of 22 different antisera on ^3H -DHT binding to ABP (abscissa) and TeBG (ordinate). Antisera diluted 1:50 (v/v) were added to an equal volume of diluted rete testis fluid (1:20, v/v) or immature rabbit serum (dilution 1:20, v/v). Normal guinea pig serum served as control. After incubation for 48 h at 2–4 °C, the samples were centrifuged at 10,000g for 15 min and binding of ^3H -DHT to ABP and TeBG measured by steady-state polyacrylamide gel electrophoresis¹⁵. No visible precipitation was seen with any of the antisera used. Nine antisera caused no inhibition of ABP and TeBG binding. Six antisera inhibited completely binding both ABP and TeBG. Seven antisera caused partial inhibition of both ABP and TeBG. Correlation coefficient for regression line = +0.985.

Thus, the present study shows that ABP and TeBG, in addition to having very similar physicochemical properties also show similar if not identical antigenicity. ABP is produced in the Sertoli cells and secreted into testicular fluid while TeBG is present in the systemic circulation but of unknown origin. It is not likely that ABP originates in serum since proteins of this size cannot penetrate the blood–testis barrier¹⁶. ABP is also found in high concentrations in the rat, which lacks serum TeBG. Furthermore, Sertoli cells in culture release ABP into the medium and this process is stimulated by FSH^{17,18}. It is also unlikely that TeBG originates in the testis, since high concentrations of TeBG are found in orchietomised males as well as normal females^{8–11}. Furthermore, ABP production is stimulated by FSH and androgens, while TeBG concentrations in serum are depressed by androgens and increased by oestrogens. Thus, ABP and TeBG might well be the same protein but produced at two different sites in the body and regulated by different hormonal mechanisms. Recent studies indicate possible differences in the carbohydrate portions of the ABP and TeBG molecules. TeBG is firmly bound by concanavalin A, while there is little or no binding of ABP (S.C.W. *et al.*, unpublished). These studies suggest minor differences in the terminal sugars of the two proteins, and chemical analysis of purified preparations will be required to establish these differences further.

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DNA replication and accelerated chain growth in oestrogen-stimulated uterine cells

OESTROGEN has been shown to reduce S phase duration in uterine epithelial cells, although no agreement has been reached either on the exact values of cell cycle parameters, or even on the relative magnitude of the hormone-induced modifications¹⁻⁶. It has also been shown that eukaryotic DNA molecules consist of replicative units arranged end-to-end, from the middle of which DNA synthesis proceeds bidirectionally at divergent fork growing points until fusion occurs with adjacent sections. The chain growth rate as well as the mean distance between initiation points were found to be specific for each cell type⁷⁻¹³. From this it can be inferred that oestrogen-dependent shortening of S phase in the uterine epithelium is due to speeding-up of replication along pre-existing units, or to multiplication of simultaneously operating replicative sections, or to both these mechanisms. The results that we have obtained from autoradiography of DNA fibres confirm that oestrogen increases the rate of DNA fork replication.

The microscopic measurements of grain tracks corresponding to replicated sections of DNA double helices show that with both ³H-TdR pulse durations uterine epithelial DNA from oestradiol-treated mice contains labelled segments which are 50% longer than in controls (Figs 1 and 2). On the other hand, distances between centres of adjacent grain tracks do not seem to vary. Only in the experimental group given a 60-min labelling pulse were the values apparently somewhat higher. Although the comparison between experimental and control data is meaningful in terms of modifications of replication rate and density of replicative units, the calculation of true fork growth rate and initiation intervals from our data presents some difficulties which have already been partly discussed^{12,13}. The histograms of track lengths are, in fact, an indeterminate mixture (see Fig. 3) of: (1) short sections arising from unidirectional replication of half-replicative units in which DNA synthesis was already under way at the start of pulse labelling; (2) labelled sections of short to medium length corresponding to bidirectional chain growth in units initiated at different times in the pulse periods; (3) average to long tracks due to fusion of adjacent replicons.

Frequencies of short tracks are probably overestimated since such segments are more often scored in the numerous short internal autoradiograms available for measurement.

Being more prone to be broken or blurred by superposition with other fibres, suitable long tandem arrays are less often found. Consequently, fork replication rate, derived from halving the modal length of labelled segments is bound to be too low. For similar reasons centre-centre distances do not represent the true value of intervals between initiation points. Measurements involving fused sections tend to overestimate the interval length whereas those performed between partly labelled halves of the same replicon will strongly underestimate it. Moreover, if two neighbouring initiation points are far apart they will be less often identified as parts of the same fibre¹¹. Therefore, although on a relative basis, centre-centre measurements do reflect the density of replicative units along DNA molecules, in absolute terms they are likely to provide a gross exaggeration of this parameter.

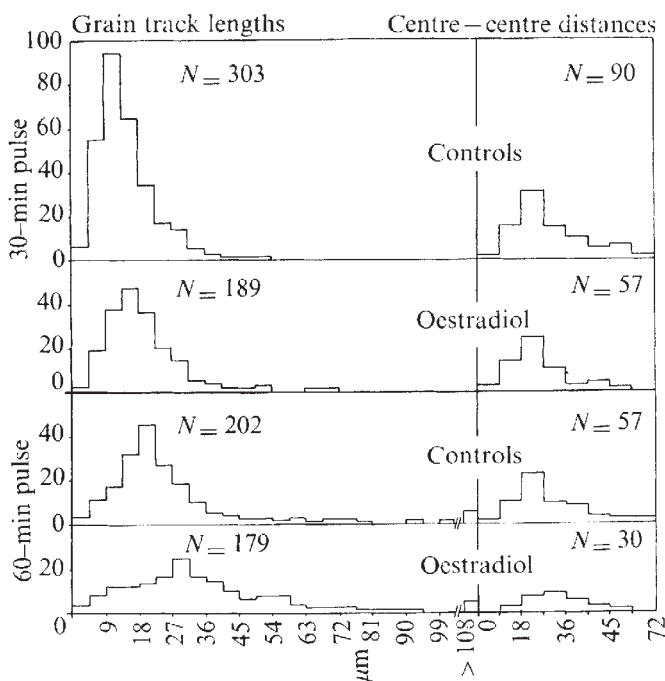


Fig. 1 Effect of oestradiol on lengths and distances between centres of ³H-TdR labelled segments in DNA molecules isolated from mice uterine epithelial cells. One week after ovariectomy, albino mice were grouped into 10 experimental and 14 control animals which were injected intraperitoneally with 100 ng 17 β -oestradiol in 100 μ l propylene glycol, respectively, or with the same volume of vehicle solvent alone. After 15 h oestrogen action the uterine horns were dissected out, split longitudinally and transferred into TC medium Eagle BSS (Difco) at 929 mg % in water, neutralised at pH 7.3 by 5% NaHCO₃ and supplemented with calf serum (10 ml per 100 ml of initial solution). A first incubation period of 30 min at 37 °C was effected in the presence of 2×10^{-6} M 5-fluorodeoxyuridine (FUDR) only, to enable optimal DNA labelling¹⁴. Thereafter the uteri were pulse-labelled in the same medium containing FUDR but supplemented with ³H-thymidine (³H-TdR) of high specific activity (52 Ci mmol⁻¹) at a final concentration of 5×10^{-6} M. In each group half of the uterine horns were removed and washed after 30 min whereas the remaining material was incubated with the isotope for 60 min. After termination of the ³H-TdR pulse, uterine epithelial nuclei were isolated as described by Smith *et al.*¹⁵. The nuclear pellets were resuspended in 0.3 ml of phosphate buffered saline (pH 7.4) and were used to perform DNA fibre smears according to the method of Lark *et al.*¹⁶. These smears were processed for autoradiography by dipping in 50% diluted Ilford K5 emulsion and were developed in 50% Dektol (Kodak) after 17 weeks' exposure. Measurements were performed with a micrometric scale only on internal autoradiograms to avoid broken DNA sections (that is by disregarding both end segments of tandem arrays on which measurements were effected). Statistical comparison was performed by use of Wilcoxon's rank test. With both pulse durations the increment of track lengths due to oestradiol was highly significant ($P < 0.001$) whereas there was no significant difference between distributions of centre-centre distances.

Our data support the conclusion that oestradiol accelerates fork growing rate. The lengthening of labelled segments from experimental uteri does not seem attributable to an increased number of fusions between neighbouring units as, first, in the 30-min pulse histogram, at least, there is no significant increase compared with controls of long fused sections, and second, there is apparently no shortening of intervals which could account for a higher rate of fusion. On the contrary, when pulse labelling of oestrogen-treated material lasted 60 min, the longer size of labelled sections induced artificial lengthening of centre-centre distances as a result of more numerous fusions which are represented by the greater right-hand tail of the corresponding histogram of track lengths. There is, however, a possibility that in addition to increasing replication speed, oestradiol would induce a higher density of operating replicative units which could not be evidenced in our study because of the relative scarcity of centre-centre measurements.

Several authors¹⁻⁶ have proposed a reduction of S phase duration in uterine epithelial cells from oestrogen-treated mice ranging from 30 to 60%. In our study the 50% increase of fork growing speed accounts for a 33.3% shortening of S phase. Only a tentative estimate of fork growth rate can be derived from our data. The modal values which were found may be assumed to lie somewhere near unidirectional growth rate since many labelled sections correspond to half replicative units. Moreover, among

Fig. 2 Cumulated frequency curves and modal values of labelled DNA lengths (log scale) in uterine epithelial cells from control and oestradiol-treated mice. Oestradiol produces a 50% increase in chain growth with both durations of ³H-TdR pulse performed on uteri incubated in vitro. Controls: Δ , 30-min pulse; $\blacktriangle$, 60-min pulse. Oestradiol: $\circ$, 30-min pulse; $\bullet$, 60-min pulse.

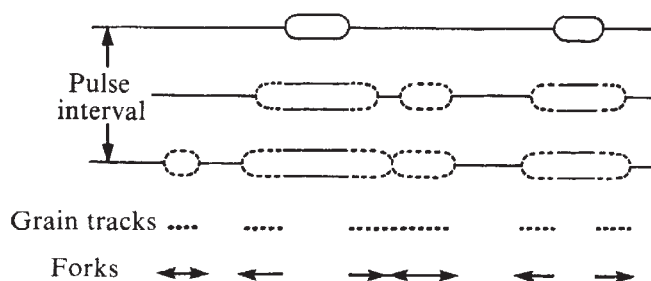
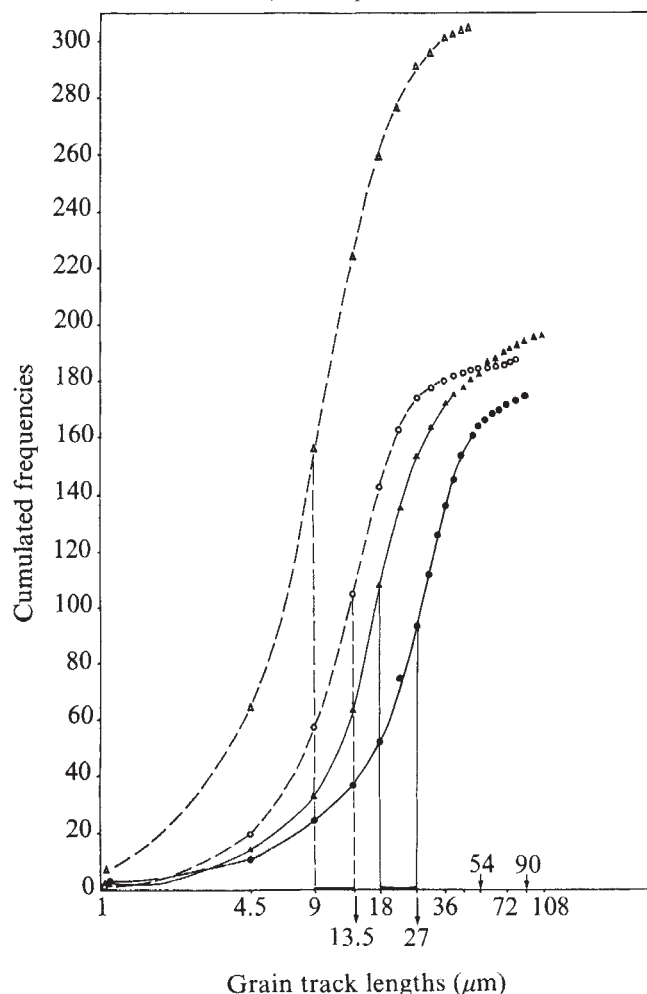


Fig. 3 Schematic example showing how different types of DNA labelled segments can be obtained after single pulse labelling with ³H-TdR (labelled sections in dotted lines). Two replicative units were already operating before giving the pulse whereas two others were initiated at the beginning and at the end of the pulse interval (modified after Blumenthal *et al.*¹³).

replicons initiated during the pulse interval, only those in which DNA synthesis started during the first half of the labelling period will show continuous grain tracks longer than monodirectional segments. True fork growth rate should therefore be evaluated at about 0.3 μm and 0.45 μm per min in controls and oestradiol-treated cells respectively. Such values would fall within the range which has been observed in other mammalian cells of which DNA growth rate has been estimated to be around 0.5 $\mu\text{m min}^{-1}$ (ref. 12).

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Troponin C in brain

THE tropomyosin-troponin complex renders actomyosin from vertebrate skeletal muscle calcium sensitive. Troponin itself consists of three subunits: troponin I (TNI), troponin C (TNC) and troponin T (TNT)¹. Pure TNI (24,000-dalton subunit) inhibits the actin-myosin interaction irrespective of calcium concentration. TNC (18,000-dalton subunit) binds calcium and releases the TNI imposed inhibition. TNT (37,000-dalton subunit) attaches the subunit complex to tropomyosin. Actin, myosin and tropomyosin have been isolated and characterised from a number of non-muscle cells^{2,3}. We now describe the isolation and characterisation of a protein from chick embryo brain which is very similar to muscle TNC.

The isolation of this molecule from brain followed our observation that an 8 M urea extract of chick embryo brain

contains a protein which migrates rapidly towards the anode in a 6 M alkaline urea acrylamide gel when 5 mM EGTA, a calcium chelator is present (Fig. 1b). The protein band has the same mobility as chicken skeletal muscle TNC (Fig. 1c). When 5 mM Ca^{2+} is added to the extract, the fast moving band disappears (Fig. 1a). This behaviour is similar to that of skeletal muscle TNC, which forms a complex with TNI in 6–8 M urea when calcium is present. EGTA blocks the complex from forming, allowing TNC to migrate rapidly in an alkaline urea gel, whereas the complex migrates much more slowly⁴.

We isolated the most rapidly migrating band from a preparative alkaline urea slab gel of a chick brain urea EGTA extract, and analysed the protein by acrylamide gel electrophoresis. The isolated protein was composed of two major polypeptides with molecular weights of 21,000 and 17,000 (Fig. 2a). A sample of chicken skeletal muscle TNC (18,000 dalton) is shown in Fig. 2b.

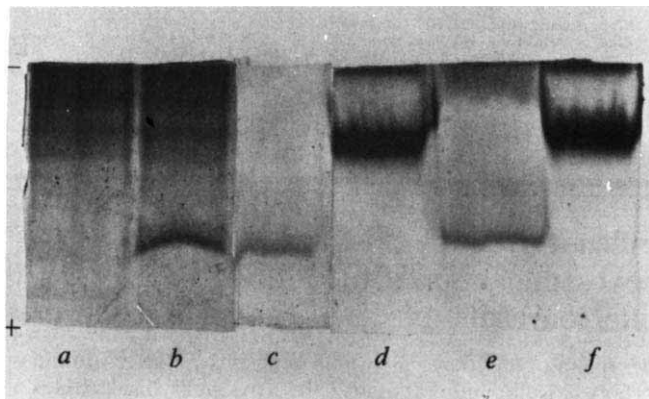


Fig. 1 Alkaline urea-acrylamide slab gels of proteins subjected to electrophoresis as described by Perry *et al.*⁴ and stained with naphthalene black. One 14-d-old chick embryo brain from which all visible blood vessels were removed was placed in two volumes of 9 M urea. The brain was then homogenised by 15 strokes in a Dounce homogeniser with a tight pestle. The solution was then centrifuged for 30 min at 100,000g and the soluble extract divided into two parts. *a*, Electrophoresis of 0.3 ml of chick brain urea soluble extract in 5 mM CaCl_2 ; *b*, electrophoresis of 0.3 ml of extract in 5 mM EGTA; *c*, 5 μg chicken skeletal muscle TNC purified as described by Greaser and Gergely¹; *d*, 5 μg of presumptive chick brain TNC and 15 μg of rabbit skeletal muscle TNI, purified as described by Greaser and Gergely¹, dissolved in 8 M urea, 5 mM CaCl_2 and electrophoresed; *e*, as in *d* except that 5 mM EGTA was substituted for CaCl_2 ; *f*, as in *d* except that 5 μg of chick skeletal TNC was used instead of the brain protein.

To obtain a pure protein, we used another property of TNC. In the absence of TNI, TNC migrates significantly more rapidly in a alkaline urea gel in the presence of Ca^{2+} than in EGTA⁴. We therefore combined the protein obtained from four EGTA preparative gels, added Ca^{2+} and ran another preparative gel. Again we eluted the most rapidly migrating band and analysed this protein by sodium dodecyl sulphate (SDS)-acrylamide gel electrophoresis. One protein band with an apparent molecular weight of 17,000 was obtained (Fig. 2c).

To determine whether the brain protein functions like muscle TNC, we substituted it for rabbit skeletal TNC in an actomyosin ATPase assay containing tropomyosin, TNI and TNT. The results shown in Table 1 indicate that the brain protein can restore calcium sensitivity to the muscle actomyosin ATPase, presumably by interacting with TNI, thereby relieving the inhibition induced by the tropomyosin-TNI-TNT complex, when calcium is present. More brain protein than muscle TNC is required, however, to relieve the inhibitory effect, and the resulting calcium sensitivity is lower than when the muscle TNC is used. It is possible that the affinity of the brain protein for muscle TNI is lower than that of the skeletal muscle TNC, or alternatively the brain protein

may be partly denatured after the rather harsh purification procedure.

Following these assays the superprecipitates formed in the presence of Ca^{2+} were collected by centrifugation at 5,000g for 5 min. One such superprecipitate was analysed by SDS gel electrophoresis. Rabbit skeletal myofibrils containing the same amount of protein were also analysed (Fig. 2d and e). A protein band is present in the superprecipitate which comigrates with the brain presumptive TNC. This band is not present in myofibrils. This result suggests that the brain protein can bind to muscle actomyosin.

The brain protein was combined with excess rabbit skeletal TNI in 8 M urea and divided into two equal parts. Ca^{2+} (5mM) was added to one part, EGTA (5 mM) to the other and they were analysed by alkaline urea gel electrophoresis. When EGTA is present, a rapidly migrating protein, with the mobility of TNC is seen (Fig. 1e). When Ca^{2+} is present this band disappears and one sees a band which has the same mobility as a complex of rabbit skeletal TNI-TNC, and presumably represents a muscle TNI-brain TNC complex (Fig. 1d and f).

The amino acid compositions of chick embryo brain TNC and two chick muscle TNCs are shown in Table 2. TNC from skeletal and cardiac muscle has a characteristically high acidic/basic amino acid ratio and a relatively high content of phenylamine as does the brain protein.

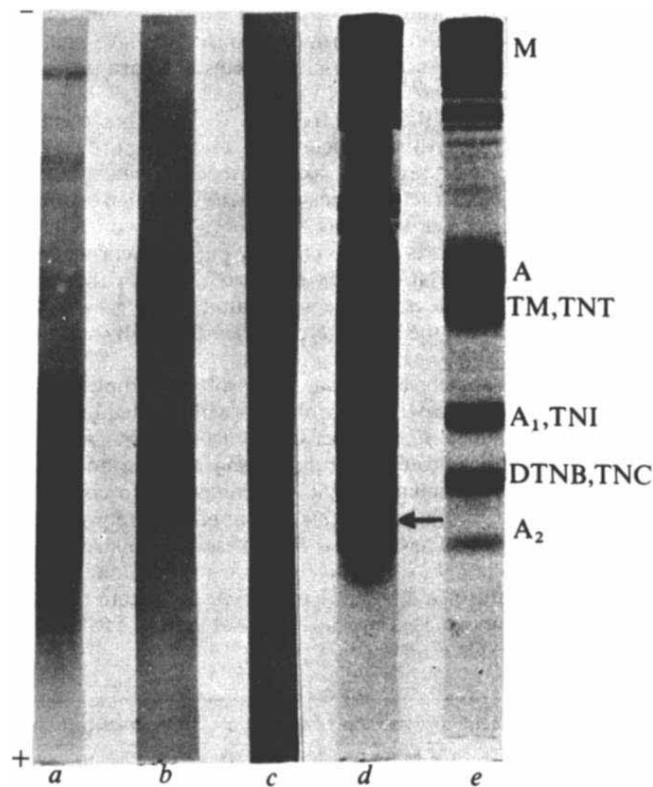


Fig. 2 10% SDS-acrylamide gels of proteins dissolved in SDS and subjected to electrophoresis as described by Weber and Osborn⁸ and stained with Coomassie brilliant blue. *a*, 25 μg of protein isolated from preparative urea slab gel electrophoresis of 6 M urea soluble chick brain extract prepared as in Fig. 1 with 5 mM EGTA present; *b*, 5 μg of chick skeletal muscle TNC; *c*, The same protein as in *a* was dissolved in 6 M urea-5 mM CaCl_2 and subjected to preparative gel electrophoresis as in *a*. 10 μg of protein eluted from most rapidly migrating band is present; *d*, Ca^{2+} -induced superprecipitate elicited by chick brain TNC. Gel was intentionally overloaded to ensure that the rapidly moving bands were visible; *e*, 200 μg of rabbit skeletal muscle myofibrils. M, Myosin heavy chains; A, actin; TM, tropomyosin; TNT, troponin T; A1, highest molecular weight myosin light chain; TNI, troponin I; TNC, troponin C; DTNB, second heaviest myosin light chain; A2, lightest myosin light chain. This system does not resolve several components into separate bands.

Table 1 Effect of chick brain troponin C on the calcium sensitivity of rabbit muscle actomyosin ATPase in the presence of tropomyosin and troponin I and T*

Troponin subunits added	Mg-ATPase ($\mu\text{mol Pi/min/mg}$ myosin)		Calcium sensitivity $\left[1 - \frac{\text{ATPase EGTA}}{\text{ATPase Ca}^{2+}}\right] \times 100$
	EGTA	Ca ²⁺	
a None	0.50	0.50	0
b Rabbit TNI-TNT	0.04	0.04	0
c Rabbit TNI-TNT + rabbit muscle TNC†	0.14	0.57	75
d Rabbit TNI-TNT + chick brain TNC†	0.06	0.11	46
e Rabbit TNI-TNT + chick brain TNC†	0.33	0.55	40

*Assays performed as described previously⁹.

†In c, 0.08 mg rabbit skeletal muscle TNC added. In d, 0.09 mg chick embryo brain TNC added. In e, 0.20 mg chick embryo brain TNC added.

These results indicate that the brain contains a protein which is chemically and functionally similar to TNC. The molecular weight of the brain protein is slightly less than that of the muscle protein suggesting either that the two proteins are specified by different genes or alternately that the proteins are products of the same gene and the brain TNC is cleaved after synthesis, or during preparation. It should be noted that brain and other non-muscle tropomyosins also have smaller molecular weights than the muscle proteins, and are products of different gene classes³.

Proteins similar to the brain troponin C are also present in growing chick sympathetic neurones, and in purified rat synaptic membranes prepared as described previously^{5,6}. In both cases a urea extract contains a protein with high mobility in an alkaline urea gel with EGTA present. The protein band disappears when Ca²⁺ is added. Partially purified neuronal and synaptic membrane TNC-like proteins also interact with muscle TNI to form complexes stable in alkaline urea gels. These findings indicate that the brain TNC is at least partially neuronal in origin.

Puszkun and Kochwa⁷ isolated a protein complex from synaptic membranes which combined with skeletal muscle myosin to produce a Ca²⁺-sensitive actomyosin ATPase. Besides containing proteins corresponding to actin and brain tropomyosin in molecular weight, the complex also contained polypeptides with chain weights of approximately 38,000, 27,000 and 20,000. They suggest that these proteins represent TNT, TNI and TNC respectively. Our results confirm the presence in brain of a TNC-like protein and indicate the presence of a protein which is similar to TNI at least in its ability to interact with TNC.

Table 2 Amino acid composition of troponin C from chicken tissues

	Chicken cardiac*	Chicken skeletal*	Chicken brain†
Lys	13	11	10
His	1	1	0
Arg	4	6	7
Asp	25	25	25
Thr	8	7	8
Ser	5	6	4
Glu	30	31	29
Pro	3	1	3
Gly	13	12	10
Ala	8	12	12
Val	8	5	9
Met	9	10	5
Ile	8	10	7
Leu	12	9	13
Tyr	3	0	2
Phe	8	11	9

*Data from Hirabayashi and Perry¹⁰.

†Analysis performed using the method of Stein *et al.*¹¹.

Results are in mol residues per 17,500 g.

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Subunit structure of human hexosaminidase verified: interconvertibility of hexosaminidase isozymes

Two major and several minor isozymes of hexosaminidase (Hex) exist in human tissues. In Tay-Sachs disease Hex A is missing but the activity of Hex B is increased. In Sandhoff's disease, both Hex A and Hex B are lacking; the residual activity represents increased amounts of a normal minor isozyme, Hex S. A fourth isozyme, Hex C, has an electrophoretic mobility very similar to that of Hex S, but seems to be unrelated genetically and structurally to A, B and S. The model which seems best to explain the relationship between Hex A, B and S is one in which they are polymers comprised of two different polypeptides which we have designated the α and β chains¹. We have proposed that Hex B is a β -chain homopolymer, Hex A an α and β -chain heteropolymer and Hex S an α -chain homopolymer². We now report that all interconversions between Hex A, B and S that are predicted by this subunit model can be performed.

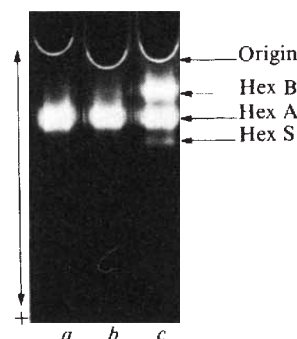


Fig. 1 Acrylamide disc electrophoresis of Hex. A: a, untreated; b, in buffered 3 M NaCl without freezing; and c, after freezing and thawing three times in buffered 3 M NaCl as described in the text. A 7.5% resolving gel in 0.375 M Tris-HCl, pH 8.9, polymerised with ammonium persulphate and TEMED, and a 2.5% concentrating gel in Tris (0.06 M)-H₃PO₄ (0.03 M), pH 7.2, polymerised with riboflavin were used. The upper buffer reservoir was Tris (0.052 M)-glycine (0.052 M), pH 8.9; and the lower reservoir was 0.1 M Tris-HCl, pH 8.1. Gels were run at 2 mA per tube for 2 h, and stained with 0.5 mM 4-methylumbelliferyl-N acetyl- β -D-glucosaminide in 0.25 M citrate-pH 4.4.

These studies were carried out with homogeneous preparations of human Hex A and Hex B, prepared by methods similar to those described previously³, but with preliminary purification of the crude placental homogenate on concanavalin A Sepharose⁴. Reactivity with antisera was determined by precipitating enzyme with antibody in a mixture containing 0.04 M potassium phosphate pH 7.0, 0.012% bovine serum albumin (hexosaminidase-free), 20 μ l of antisera⁵ or control serum and 1–5 mU of enzyme. After incubating overnight at 4 °C, the mixture was centrifuged at 40,000g for 60 min and supernatant Hex activities were determined. Assays were carried out as before³.

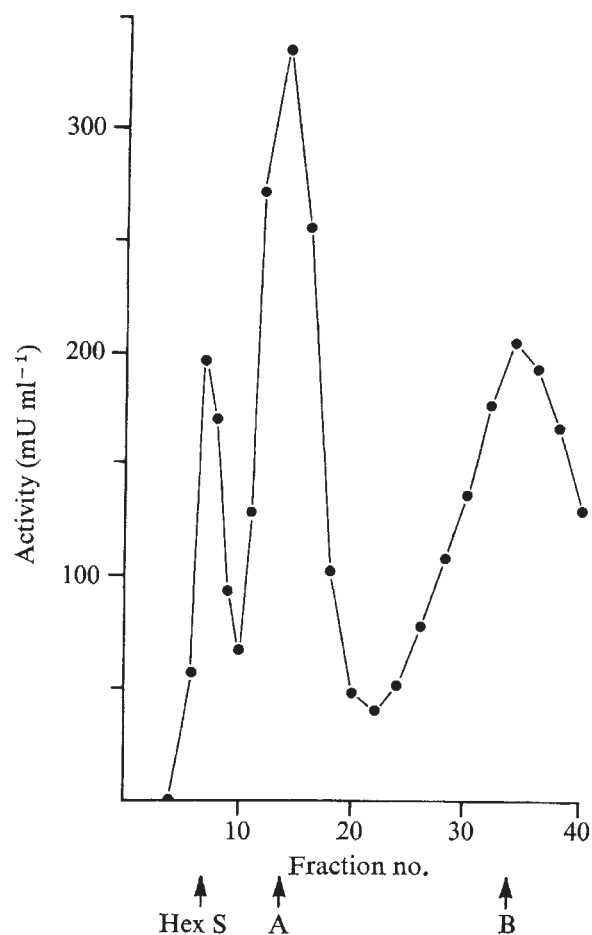


Fig. 2 Elution pattern from preparative polyacrylamide gel electrophoresis of Hex A which had been frozen and thawed in a high ionic strength salt solution. The system described for Fig. 1 was used with a Buchler Polyprep 200 apparatus with the lower buffer reservoir containing a fourfold concentrated buffer. Elution was performed with a 1:4 dilution of this buffer.

Homogeneous Hex A at a concentration greater than 4 U ml⁻¹ could be converted directly into Hex B and an enzyme indistinguishable from Hex S by freezing and thawing in 3 M sodium chloride in 0.02 M potassium phosphate buffer, pH 7.0. After three or four cycles of freezing in a dry ice-acetone bath and thawing, the enzyme was submitted to acrylamide disc electrophoresis. As Fig. 1 shows, this treatment resulted in the appearance of bands of activity in the positions of Hex B and Hex S, which seemed to reach a maximum after four cycles of freezing and thawing. The formation of Hex B and Hex S could also be demonstrated on Cellogel electrophoresis. The isozymes found in a frozen and thawed preparation were separated on preparatory acrylamide disc electrophoresis (Fig. 2). The putative Hex S isozyme isolated by this procedure was reactive with anti-Hex A (83% enzyme

removed) but showed little or no reactivity with anti-Hex B (14% enzyme removed), just as we had found with authentic Hex S obtained from tissues of a patient with Sandhoff's disease².

Since an equilibrium mixture between Hex A, B and S seemed to be formed on repeated freezing and thawing, it seemed possible that Hex A could be formed by freezing and thawing a mixture of Hex S and Hex B. As Fig. 3 shows, this was achieved using both the Hex S prepared from homogeneous Hex A (see above) and Hex S partially purified from the liver of a patient who had died of Sandhoff's disease. The Hex A formed by hybridisation of both Hex S preparations with Hex B was indistinguishable from placental Hex A by Cellogel electrophoresis and by DE 52 chromatography. No Hex A was found in a mixture of Hex B and S that was not subjected to freeze-thaw treatment, nor was any Hex A formed when Hex B or S were frozen and thawed separately.

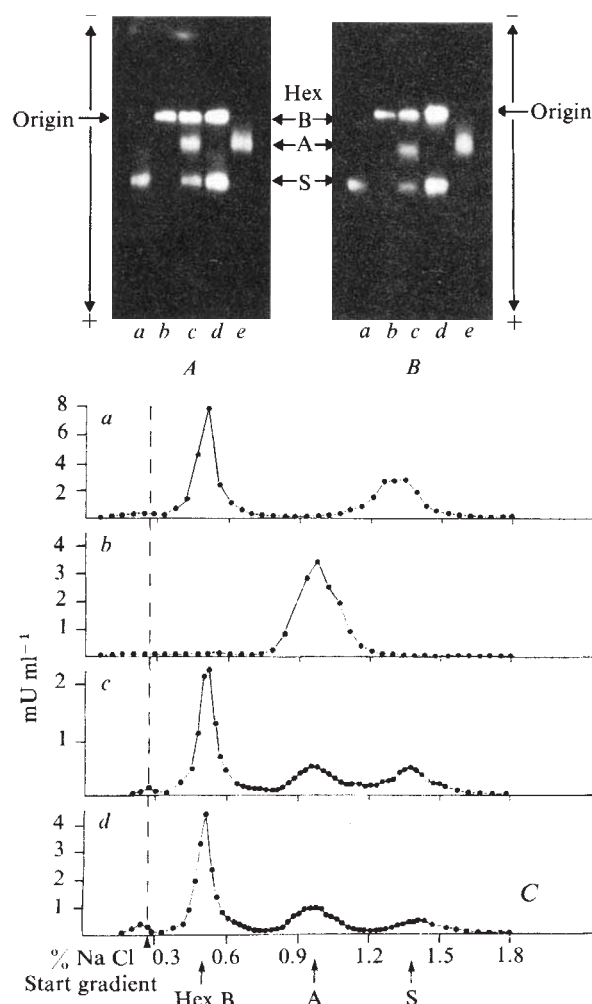


Fig. 3 A, Cellogel electrophoresis of: a, placental Hex S; b, placental hex B; and c, a mixture of placental Hex S and Hex B, all of which have been frozen and thawed three times in buffered 3 M NaCl. Channels d and e, are unfrozen controls of mixed placental Hex S and placental Hex B (d) and placental Hex A (e). The placental Hex S preparation was prepared from placental Hex A (as shown in Fig. 2). Electrophoresis was carried out for 2 h in a 20 mM potassium phosphate buffer, pH 7.0, at 92 V across the gel and 0.6 to 0.8 mA per cm. B, the same as (A) except that authentic Sandhoff's liver Hex S was used in place of Hex S prepared from placental Hex A. C, Column chromatography elution on DE 52 (ref. 18) of: a, mixture of placental Hex S and placental Hex B which was not frozen; b, placental Hex A; c, placental Hex S and placental Hex B frozen and thawed three times in buffered 3 M NaCl; and d, Sandhoff's liver Hex S and placental Hex B frozen and thawed three times in buffered 3 M NaCl.

The close relationship between Hex A and Hex B has been evident for several years: both enzymes are missing in Sandhoff's disease and the two isozymes are strongly immunologically cross reactive. Models proposed to account for this relationship include the suggestions that Hex A is merely the neuraminic acid derivative of Hex B (refs 6 and 7) that Hex A and Hex B are conformers⁸, that Hex A and Hex B are unrelated⁹, and that Hex A and Hex B have a common subunit¹⁰. The results of attempts to distinguish between some of these alternatives by studying interspecific somatic cell hybrids have been confusing. Some studies indicated that the presence of Hex B was dependent on the presence of Hex A¹¹, some indicated that Hex A formation was dependent on the formation of Hex B¹², and others that Hex A and B are unrelated^{9,13}. We believe that problems in interpretation of results of interspecific cell hybridisation arise because of difficulties in correctly identifying the enzymes in hybrid clones. Such difficulties

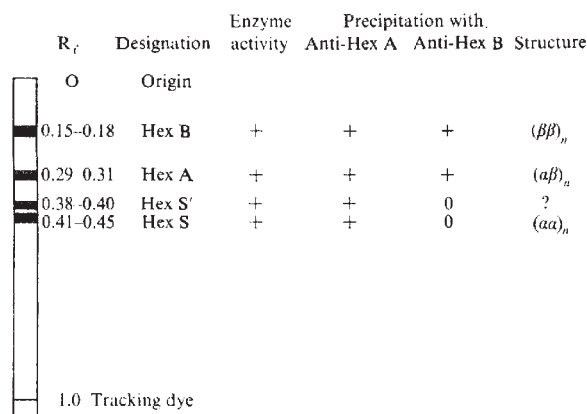


Fig. 4 Summary of properties and subunit structures of hexosaminidase isozymes

are magnified in the case of glycoproteins, because the sugars attached to the protein may vary even from tissue within the same species¹⁴.

According to the model we have proposed¹, Hex A has a polypeptide structure of (αβ)_n, Hex B, (ββ)_n, and Hex S, (αα)_n. Our model was derived from, and supported by, the following considerations: (1) the amino acid composition of homogeneous human Hex A and Hex B are different¹⁵; (2) Hex A contains an antigenic determinant not present in Hex B¹, but all the antigenic determinants present in Hex B are found in Hex A^{1,16}; (3) Hex S contained only the antigenic determinants of Hex A and not those of Hex B^{1,17}; (4) Hex A can be converted to Hex B and an alkylated α chain by treatment with merthiolate or *p*-hydroxymercuribenzoate¹⁸ (not by neuraminidase, as had been claimed); (5) hybridisation of fibroblasts from Sandhoff's disease and Tay-Sachs disease produced complementation with the formation of Hex A¹⁹. According to our model, Tay-Sachs disease represents a mutation involving the α subunit, preventing formation of Hex A, (αβ)_n, and making available excess β chains, thus accounting for the increased levels of Hex B, (ββ)_n. Conversely, Sandhoff's disease represents a mutation involving the β chain, preventing formation of Hex A and Hex B and making available excess α chains for the formation of the α-chain homopolymer, Hex S. The analogy between this model and the thalassaemias is evident: in α thalassaemia homopolymers of β chains form haemoglobin H, while free α chains are detected in β thalassaemia.

Our ability to interconvert Hex A, Hex B and Hex S demonstrates clearly the correctness of the subunit model. The properties and subunit structures of hexosaminidase isozymes are summarised in Fig. 4. Although we suggested

previously that Hex S was probably a homopolymer (αα)_n, a heteropolymeric structure, (αγ)_n, could not be ruled out in our earlier studies². But the fact that Hex S as well as Hex B can be produced from homogeneous Hex A indicates that no subunits in addition to α and β are required to account for the formation of Hex A, B and S, and that Hex S is, indeed, an α-chain homopolymer.

On the other hand, Hex C, an enzyme which has been described in some detail by Dreyfus, Robinson and others²⁰ seems to be independent of the Hex A, B and S system. We have been able to show that the residual brain enzyme of our patient with Sandhoff's disease² was Hex C, and that it could easily be distinguished from Hex S on side-to-side comparison. We have confirmed the observation that this enzyme, from normal or Sandhoff's disease brain, is not affected by antisera produced against Hex A and Hex B, nor is it precipitated by an antiserum produced against Hex S. It seems likely that Hex C is a genetically independent protein. The minor isozymes, Hex I and Hex S'² are probably merely manifestations of the microheterogeneity of the carbohydrate portion of Hex B and Hex S, respectively.

Although the nature of the C, S' and I isozymes require further study, the interconvertibility of Hex A, B and S verifies their proposed subunit structures. These findings are inconsistent with somatic cell hybridisation studies which assign totally distinct loci to Hex A and Hex B⁹ or to the suggestion that the differences between Hex A and Hex B are merely conformational⁸.

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Stepwise degradations and deamidation of the eye lens protein α-crystallin in ageing

KNOWLEDGE of the intracellular processes of ageing and breakdown of proteins is still very limited^{1,2}, but the extremely slow turnover of lens proteins in the eye provides a unique opportunity to analyse such processes. Throughout life fibre cells are formed from epithelial cells at the periphery of the lens, being successively covered by younger cells. Protein synthesis ceases soon after the differentiation into fibre cells is completed³. As a result the nucleus of the lens of an adult still contains the proteins formed during foetal life. Here we

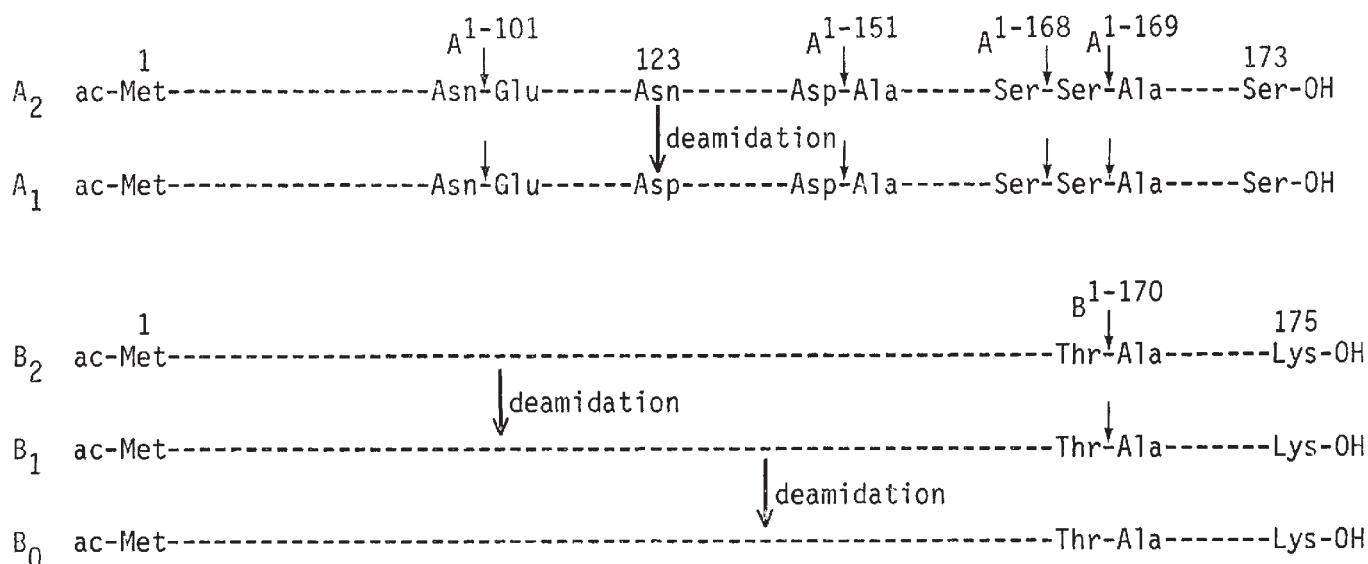


Fig. 1 Polypeptide chains of bovine α -crystallin and their postsynthetic modifications. The newly synthesised A_2 and B_2 chains are 173 and 175 residues long respectively, showing 57% sequence homology^{7,8}. Deamidation in A_2 of 123-Asn and possibly a second amide gives rise to A_1 (W. W. de J., F. J. van der Ouderaa and H. Bloemendal, unpublished). A_1^{1-169} , A_1^{1-151} and A_1^{1-168} are degradation products lacking the last 4, 5 and 22 residues of the normal A chain, and each occurs in two electrophoretic forms, corresponding to the amidated and deamidated state^{9,14}. A_1^{1-101} lacks 72 residues from the C terminus and occurs in a single electrophoretic form¹⁰. B_1 and B_0 are most probably successive deamidation products of B_2 , although the sites of deamidation have not been determined¹⁰. Removal of five residues at the C-terminal end of B_2 and B_1 gives rise to B_2^{1-170} and B_1^{1-170} (ref. 10).

demonstrate that differences in polypeptide structure observed between the cortex and nucleus⁴⁻⁶ of the lens are due to an ageing process. Our data provide the first example of progressive degradation and deamidation of a protein, probably caused by non-enzymatic mechanisms.

The polypeptide chains of the major lens protein α -crystallin, a high molecular weight aggregate, are well characterised⁷⁻¹⁰ (Fig. 1). It seems that all α -crystallin chains can be related to A_2 and B_2 by deamidation and degradation processes. The proportion of shortened chains increases from cortex to nucleus¹⁰ (Fig. 2), suggesting an age-dependent degradation process, since no protein synthesis takes place any more in the inner cortex and nucleus. These data do not, however, exclude the possibility that the shortened chains are synthesised *de novo* in embryonic lenses, and are present in older nuclei as remnants of this embryonic stage. We therefore investigated the subunits of α -crystallin from prenatal lenses.

It has been shown previously that foetal bovine lenses contain mainly A_2 and B_2 (refs 11 and 12). In very young embryos two additional subunits have been observed¹³ which we found by isoelectric focusing to correspond in position to two of the additional subunits of the older lens (Fig. 3A). The two additional embryonic subunits were isolated and analysed by tryptic digestion and peptide mapping, using previously described methods^{7,8}. The peptide maps were identical to those of A_2^{1-151} and B_2^{1-170} isolated from calf nucleus. Analysis of embryonic α -crystallin by gel electrophoresis in the presence of sodium dodecyl sulphate (SDS) revealed, moreover, the presence in large amounts of the A_2^{1-169} (or A_2^{1-168}) chain, which does not separate from normal A_2 on isoelectric focusing (Fig. 3B). Peptide mapping of a mixture

of embryonic A_2 and presumed A_2^{1-169} confirmed it to be genuine A_2^{1-169} (ref. 14).

These findings show, therefore, that the shortened chains A_2^{1-169} , A_2^{1-151} and B_2^{1-170} are already present in embryonic lenses. Are these chains then the products of independent genes or postsynthetic derivatives of A_2 and B_2 ? The absence of A_1^{1-101} in embryonic lenses proves that it is an ageing product of normal A chains. For A_2^{1-168} and A_2^{1-169} it has already been demonstrated that they are degradation products of A_2 (ref. 14). Both A_2^{1-151} and B_2^{1-170} are present in higher proportions in adult nuclear α -crystallin than in embryonic α -crystallin, increasing from 7 to 25%, and from 5 to 15%, respectively.

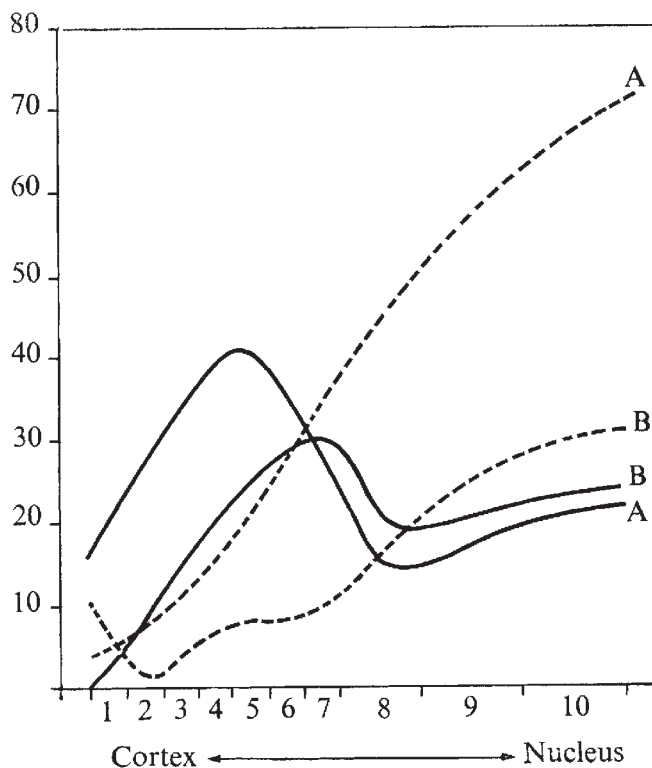


Fig. 2 Ratios of degraded (-----) and deamidated (——) A and B chains in α -crystallin from successive concentric layers of calf lens (22 weeks old). α -Crystallin was isolated from ten concentric layers, corresponding to the numbers indicated on the abscissa, and analysed by acrylamide isoelectric focusing. The proportions of the different polypeptide chains were determined by scanning of the stained gels. The values on the ordinate express the percentage of degraded and deamidated A and B chains relative to the total amount of A and B chains, respectively.

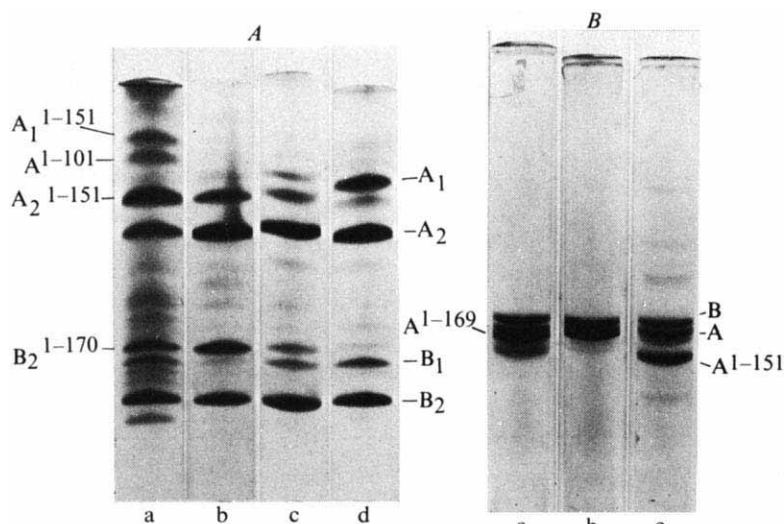


Fig. 3 A, Isoelectric focusing gels (pH 5-8) in 6 M urea of α -crystallin from: a, calf lens nucleus; b, 3-month-old foetus; c, 6-month-old foetus; d, calf lens cortex. B, SDS gel electrophoresis of α -crystallin from: a, 3-month-old foetus; b, calf lens cortex; c, calf lens nucleus.

This indicates that the formation of these chains continues in the adult nucleus, which is devoid of biosynthetic activity, and thus demonstrates the postsynthetic origin of these chains. Further proof comes from the fact that A¹⁻¹⁵¹ and B¹⁻¹⁷⁰ can be produced *in vitro* simply by prolonged dialysis of α -crystallin against 0.1 M Tris-HCl buffer, pH 7.3 (ref. 15).

Our data thus show that the A₂ and B₂ chains are subjected to two types of postsynthetic modifications: deamidation and C-terminal degradation, as summarised in Fig. 1. The deamidation products A₁ and B₁ only appear in the later stages of foetal life and reach their highest level in the old bovine cortex, where a probable further deamidation product also becomes prevalent: B₀ (ref. 10). Postsynthetic deamidations have been demonstrated in other proteins, and can be obtained *in vitro* in proteins and synthetic peptides¹⁶. This deamidation process is non-enzymatic and its rate depends on the character of neighbouring residues¹⁶.

The degradation of α -crystallin chains is apparently a post-synthetic process which starts early in embryonic life. The number of degraded chains is, however, clearly largest in the old bovine nucleus¹⁰. It seems, therefore, justified to consider these degradations as an ageing process. It seems that the chains are preferentially cleft at the sites indicated in Fig. 1. A stepwise breakdown, degrading the A chain successively to 169, 168, 151 and 101 residues, is suggested by the very high level of A₂¹⁻¹⁶⁹ and the absence of A¹⁻¹⁰¹ in the embryonic lens, and the low level of A¹⁻¹⁹⁸ and high level of A¹⁻¹⁰¹ in the old bovine nucleus¹⁵.

Several features make it unlikely that the observed degradations are the result of enzymatic processes. The appearance of A¹⁻¹⁰¹ is a very slow process, being most pronounced in the nucleus, which implies that a putative enzyme becomes active only very late after its synthesis. If the enzyme were an endopeptidase it should have a specificity (compare Fig. 1) unlike any known endopeptidase. If it were an exopeptidase it should have carboxypeptidase C-like activity to remove proline residues, and be delayed at the sites indicated in Fig. 1. The fact that degradations can be caused by prolonged dialysis of purified α -crystallin would require that the possible enzyme has the same molecular weight as α -crystallin or that the degradation is an autolytic process. Also the action of lysosomal enzymes cannot be involved, since no lysosomes seem to be present in the nucleus of the lens¹⁷. In conclusion we propose that the observed degraded chains are the result of non-enzymatic breaks of susceptible bonds.

The knowledge obtained from the age-related phenomena in eye lens proteins may contribute to a better understanding of ageing in general; although it can be questioned whether the processes taking place in the ageing lens are representative of ageing in other types of cells. In addition, the knowledge of normal ageing of lens proteins is of great interest to the study of those forms of cataract which are considered to be the

result of precocious ageing¹⁸.

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Erratum

In the article "Discrimination between eukaryotic and prokaryotic, and formylated and non-formylated, initiator tRNAs by eukaryotic initiation factor EIF-3" by I. G. Wool and R. S. Ranu (*Nature*, **257**, 616; 1975), the first four lines should read

PROKARYOTIC and eukaryotic cells contain two methionine accepting species of transfer RNA, tRNA_{Met}^f and tRNA_{Met}^{Met} (refs 1-3). The formylated Met-tRNA_f (fMet-tRNA_f) in prokaryotes⁴ and non-formylated Met-tRNA_f from . . .

and not as printed.

matters arising

Sympathoadrenal medullary activity in young, spontaneously hypertensive rats

INCREASED levels of serum dopamine- β -hydroxylase (DBH) have been reported in spontaneously hypertensive rats (SHRs)¹. Although DBH is released from sympathetic nerve endings along with noradrenaline, levels of the enzyme in plasma do not seem to reflect accurately the degree of sympathoadrenal activity². Plasma levels of noradrenaline mostly reflect sympathetic neuronal activity whence adrenaline (A) levels are indicative of adrenal medullary discharge. The content of catecholamines and biosynthetic

were derived were used as controls. Activities of adrenal tyrosine hydroxylase (TH), DBH, and phenylethanolamine-N-methyl transferase (PNMT), as well as plasma DBH, were also examined.

Male rats, 4 weeks old and weighing 70–90 g, were decapitated, and the first 1.5 ml of blood, which was ejected in a pulsatile stream from the trunk, was collected in heparinised tubes kept on ice. After centrifugation at 6,000g for 20 min at 4 °C, an aliquot of the plasma was removed for an assay of the DBH activity⁴. The proteins in 400 μ l of the plasma were precipitated by the addition of 10 μ l of 40% perchloric acid, and the mixture was kept at –10 °C until it was assayed for total catecholamines (adrenaline + noradrenaline)⁵ and noradrenaline^{6,7}.

TH, DBH, and PNMT in the adrenals of 4-week-old SHR were decreased (Table 2). Furthermore, the A content of the adrenals of the SHRs were lower than those of the WKY.

These observations are consistent with the view that, at 4 weeks of age, sympathetic neuronal activity in SHRs is increased, whereas adrenal medullary activity is depressed. Adrenal medullary activity is increased when sympathetic nerves are destroyed by 6-hydroxydopamine¹² and it is likely that depressed adrenal medullary activity may be secondary to the increased sympathetic neuronal activity. The exaggerated sympathetic neuronal activity at that age may be an essential factor in the course of development of hypertension in SHRs.

During this study, H.G. was supported by the Deutsche Forschungsgemeinschaft, Bonn, West Germany. J.M.S. is a visiting scientist at the National Institute of Mental Health, and M.F.R. is a research associate in the Pharmacology-Toxicology Program of the National Institute of General Medical Sciences.

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Table 1 Catecholamine concentrations* and dopamine- β -hydroxylase activity* in the plasma of 4-week-old spontaneously hypertensive and normal rats

	WKY	SHR
Total catecholamines	11.9 $\pm$ 0.07	13.2 $\pm$ 0.8
Noradrenaline	1.8 $\pm$ 0.14	2.93 $\pm$ 0.35†
Dopamine- β -hydroxylase	7.84 $\pm$ 0.24	13.4 $\pm$ 0.6†

* Results expressed as ng of catecholamines, ng of noradrenaline and units of enzyme per ml of plasma, and are the means ($\pm$ s.e.m.) for groups of 14 animals.

† $P < 0.001$ compared with control animals.

‡ $P < 0.01$ compared with control animals.

Table 2 Catecholamine content* and biosynthetic enzyme activities* in the adrenal glands of 4-week-old spontaneously hypertensive and normal rats

	WKY	SHR
Tyrosine hydroxylase	11.9 $\pm$ 0.48	8.67 $\pm$ 0.24‡
Dopamine- β -hydroxylase	25.6 $\pm$ 2.6	17.7 $\pm$ 0.92‡
Phenylethanolamine-N-methyl transferase	8.43 $\pm$ 0.18	5.13 $\pm$ 0.12†
Adrenaline	2.15 $\pm$ 0.15	1.70 $\pm$ 0.09§
Noradrenaline	0.85 $\pm$ 0.10	0.75 $\pm$ 0.04

* Results are expressed as ng of catecholamines, ng of noradrenaline, and units of enzyme per mg of adrenal and are the mean values ($\pm$ s.e.m.) for groups of 14 animals.

† $P < 0.001$ compared with control animals.

‡ $P < 0.01$ compared with control animals.

§ 0.02 < $P < 0.05$.

enzymes in the adrenal are presumably indices of the activity of the adrenal medulla in synthesising and releasing catecholamines. The possible role of the sympathoadrenal system in initiating the increase in blood pressure in SHRs was examined by measuring levels of adrenaline and noradrenaline in the plasma and adrenal glands at the age of 4 weeks, the age at which the blood pressure of SHR begins to rise³. Normotensive rats of the Wistar Kyoto strain (WKY) from which the SHRs

The catecholamine content of the adrenals was assayed fluorimetrically⁸, and the TH (ref. 9), DBH (ref. 4), and PNMT (ref. 10), activities were assayed radiometrically.

In young SHRs the plasma levels of both the noradrenaline and the DBH were increased over those of the WKY (Table 1) but the total catecholamines (mostly A) were not significantly different. In contrast to the increased TH and DBH activities in the adrenal glands of adult SHR¹¹, the activities of

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NAGATSU REPLIES—The findings of Grobecker *et al.*¹ agree with previous results² on increased serum DBH of SHRs at 3 weeks of age, and are consistent with the view that sympathetic neuronal activity in young SHRs is increased. As additional evidence, I have found that DBH activity in the mesenteric vessels of SHR at 3 weeks of age is

increased, indicating increased sympathetic neuronal activity in blood vessels. Interestingly, DBH activity in the locus coeruleus area of the brain is decreased³.

In contrast, serum DBH activity and tyrosine hydroxylase (TH) and DBH activities in blood vessels of SHR rats at 14 weeks of age do not differ significantly from those of normotensive Wistar-Kyoto rats⁴, whereas TH and DBH activities in adrenal glands are increased^{4,5}. These results suggest that the increased sympathetic neuronal activity in young SH rats is decreased during the development and establishment of hypertension in adult SHRs and whereas the adrenal medullary activity in adult SHRs is increased.

The results of Grobecker *et al.* on decreased TH, DBH, and phenylethanolamine N-methyltransferase (PNMT) in the adrenal glands of SHRs at 4 weeks of age are of interest and indicate depressed adrenal medullary activity in contrast to increased sympathetic neuronal activity. I have, however, observed a slight but significant increase of DBH activity in the adrenal glands of SHRs at 3 weeks of age⁸.

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Can supernovae make white dwarfs?

LEVENTHAL and McCall¹ have presented a model for a type I supernova light curve, in which the two sections of exponential decay of the light curve are explained by the accelerated decay of $^{56}\text{Ni} \rightarrow ^{56}\text{Co} \rightarrow ^{56}\text{Fe}$, occurring in a fully convective white dwarf which is formed by the supernova implosion-explosion. While they did give reasons why a type I supernova would be expected to synthesise ^{56}Ni , they failed to discuss whether it would produce a white dwarf, a result which is strongly, though not entirely, unsupported by recent calculations.

The problem centres on whether the ^{12}C -detonation model of a supernova, thought to apply to stars in some sub-range of $4-8M_{\odot}$, can leave a white dwarf, or, indeed, any collapsed remnant. It is already clear (ref. 2 and W. D. Arnett, unpublished) that stars more massive

than $\sim 8M_{\odot}$ all undergo core collapse and produce neutron stars or black holes.

Buchler³ has assumed various arbitrary central densities, higher than those now thought to be attainable, for half ^{12}C , half ^{16}O , $1.4M_{\odot}$ degenerate cores and calculated the results for carbon detonations at these densities. At central density $\rho_c = 8.6 \times 10^9 \text{ g cm}^{-3}$, the star was completely disrupted. In a small range around $\rho_c = 1.6 \times 10^{10} \text{ g cm}^{-3}$, his calculation did in fact produce an extended $1M_{\odot}$ Ni-Fe white dwarf, with the remaining $0.4M_{\odot}$ being blown off. At $\rho_c = 2 \times 10^{10} \text{ g cm}^{-3}$ a neutron star remnant was produced.

Couch and Arnett⁴ have recently carried out further calculations, using the ^{12}C detonation model and including all effects yet thought of, such as a convective Urca shell, which might postpone carbon ignition and allow the high central densities necessary for collapsed remnant formation to be achieved. Their models disrupted completely, except for the case where the C-O core was initially only 10% C by mass. The carbon burned non-explosively and a remnant could possibly be formed unless the central density reached a critical density (between 1×10^{10} and $2 \times 10^{10} \text{ gm cm}^{-3}$), where the oxygen would ignite. They point out however that if He burning had produced such a low C-O ratio, it might become impossible to understand the nucleosynthesis of C and O, which is, presumably, done in higher mass stars, which are able to preserve and eject these nuclei.

Thus the formation of white dwarfs by supernovae remains highly problematical. If it could be shown to occur, Leventhal and McCall's model of type I supernova light curves would gain much credibility. It could also frustrate attempts (ref. 5 and B. M. Tinsley, unpublished) to deduce the mass below which, stars are able to shed enough mass by the planetary nebula mechanism to become stable white dwarfs, by counting white dwarfs in open clusters with high-mass turn off points on the main sequence.

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Carotid body chemoreceptors

THE "New theory for receptor mechanism of carotid body chemoreceptors" proposed by Osborne and Butler¹ requires that the rate of dopamine secretion from the glomus cells be high when blood gas tensions are normal and reduced in hypoxic conditions. The results of new experimental studies^{2,3} are incompatible with the 'new theory' because the relationship between secretion of carotid body catecholamines and blood oxygen was found to be opposite to that postulated. In one study², the formaldehyde-induced fluorescence of the glomus cells was measured to monitor changes in their catecholamine content. In untreated cats, hypoxia resulted in higher fluorescence intensity of type I cells from innervated carotid bodies than from denervated ones. When cats were treated with a decarboxylase inhibitor, intensity of fluorescence was decreased in innervated carotid bodies compared with denervated organs after hypoxia. Sampson *et al.*² concluded that an increase in centrifugal impulse activity in the sinus nerve induced by hypoxia enhanced both release and synthesis of catecholamines in the carotid body. In the second study³, the content of noradrenaline plus adrenaline in the cat carotid body was measured by the trihydroxy indole method. The content of these amines in the carotid body decreased after hypoxia and increased above the air control level when inspired oxygen concentration was raised to 40%. The decrease in catecholamine content was greatly attenuated when the carotid sinus nerve was cut before hypoxia.

Since the fluorescence technique used measured dopamine as well as noradrenaline and adrenaline, the increase in fluorescence in untreated carotid bodies after hypoxia may be attributed to enhanced synthesis of dopamine, which serves as precursor for noradrenaline and adrenaline. This is the case in the adrenal medulla where neurogenic stimulation activates tyrosine hydroxylase and increases dopamine content while releasing noradrenaline and adrenaline⁴. The results of the two carotid body studies^{2,3} are consistent with the idea that stimulation of efferent neural pathways to the carotid body during hypoxia enhances both synthesis and release of catecholamines. The data provide no support for the 'new theory'¹ which requires that hypoxia depress secretion from the glomus cells of the carotid body and postulates that secretion is regulated independently of sinus nerve efferent pathways.

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OSBORNE AND BUTLER REPLY—Our conclusion¹ that a catecholamine (probably dopamine) is continuously released from the glomus cells during normoxia, and that the rate of release decreases during hypoxia, was based on the following: (1) glomus cells store large amounts of catecholamines, in particular, dopamine²; (2) catecholamines, especially dopamine, suppress or block the chemoreceptor discharge^{3,4}; and (3) α -adrenoreceptor blocking agents greatly increase receptor firing rate (up to tenfold)^{3,4} during normoxia and the effectiveness of these blocking agents decreases during hypoxia⁵.

It has since been indicated that there is a high turnover rate of catecholamines in the glomus cells, and that it seems likely that these cells release dopamine at a high rate⁶. This catecholamine activity by the glomus cells is envisaged to occur in normoxic conditions. Indeed, the metabolism of catecholamines requires the presence of molecular oxygen⁷. These observations suggest that as the oxygen availability decreases, the metabolism of catecholamines declines.

In the above article⁷, the phrase "the increase in fluorescence in untreated carotid bodies after hypoxia" is puzzling in view of the facts that Mills and Slotkin⁸ describe a decrease in catecholamine content of the carotid body during hypoxia, and Sampson *et al.*⁹ did not in fact make the straightforward comparison between the amounts of catecholamines present in the glomus cells of untreated normoxic and hypoxic carotid bodies. We do not therefore accept that these two papers^{8,9} contain unequivocal evidence regarding the effect of hypoxia on the release of catecholamines from the glomus cells of the carotid body.

Furthermore, Mills and Slotkin⁸ state that the decrease in catecholamine content of the carotid body during hypoxia is largely mediated by way of the sinus nerve, whereas Sampson *et al.*⁹ report that the catecholamine

content of the glomus cells of the carotid body was higher in those organs with an intact sinus nerve. In an attempt to reconcile these apparent differences, Mills *et al.*⁷ state more precisely that Mills and Slotkin⁸ were referring to noradrenaline and adrenaline only, whereas Sampson *et al.*⁹ measured total catecholamines. They now conclude that dopamine levels are increased within the glomus cells, whereas noradrenaline and adrenaline are released in response to efferent nerve activity. Whether this effect is mediated through a direct supply to the glomus cells or via the vasculature is not discussed by these authors, but it does raise the important issue regarding which catecholamine(s) is the major agent of chemoreceptive inhibition.

As has been shown^{8,10,11}, and as pointed out here, dopamine seems to be the favoured candidate.

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Spectacle haloes

THE nuclear geophysical enigma of the ²¹⁰Po haloes¹ is quite fascinating, but the explanation put forward on the basis of a novel form of ²⁰⁸Pb is not easy either to understand or to believe.

If no primordial lead is present, all of the ²⁰⁶Pb, ²⁰⁷Pb and ²⁰⁸Pb found in ordinary U–Th haloes will be derived from polonium α decay. To explain all of the polonium haloes observed by Gentry^{1–3} without apparent α -emitting precursors it is not useful to assume new types of stable lead daughter products but rather some unknown, long lived isomers either of ²¹⁸Po and ²¹⁰Po or of β -emitting precursors of these. The haloes may be explicable without such an assumption. What is needed is that in a usual halo the polonium isotopes must decay while still in the uranium inclusion from which they are derived, whereas in the enigmatic halo they must together have migrated by at least hundreds of micrometres from their ancestral uranium.

Since the enigmatic halo has an intensity comparable with that of an ordinary halo, it must be attributable to the decay of quantities of polonium corresponding

to the total output of a normal, halo-producing, uranium inclusion for at least tens of millions of years. That is a very long period compared with the half lives of isotopes of any elements other than uranium and thorium themselves. Conditions must, therefore, have been such as to allow migration from the original parent sources for very long periods.

The most likely migrant would seem to be lead, since emanation or polonium would give larger haloes than are seen. In that case there would be ²¹⁰Pb with a half life of 22 yr in one series and ²¹¹Pb with a half life of 38 min in the other. Further, the ²³²Th series, which eventually produces the observed ²⁰⁸Pb, has ²¹²Pb with a half life of 10.6 h as a carrier, though it has no polonium with a half life of longer than 0.16 s. I do not know the temperature required for that migration of lead at a suitable rate, but it is, presumably, fairly high. Geological evidence would be interesting: Gentry himself has described² short fission tracks which suggest that some mica matrices may have been at a high temperature for a long time.

I do not claim that this explanation is highly probable—the events to be explained are rare—but Gentry's explanation would seem to require a higher order of improbability. Both ²¹⁸Po and ²¹⁰Po would need to have unknown precursors with half lives of a few hundred million years; lead or bismuth precursors would presumably have crystallised out with observable quantities of stable isotopes of the same elements. Finally, Gentry has also found^{2,3} a variety of dwarf or other haloes for which he assumes different, otherwise unknown, α -emitters. All of those α -emitters apparently have mutually similar half lives or they would either have decayed too soon or would still be around in observable amounts. One such unknown isomer would be unlikely enough; so many would seem very close to impossible.

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GENTRY REPLIES—The crucial question is whether the polonium atoms which formed the polonium haloes were secondarily derived from the decay of uranium and thorium (see, for example, ref. 1) or from unknown isomers¹, or whether they were primordial.

The isomer hypothesis¹ is one way that polonium haloes could have been explained. My present view is that experimental results have ruled out the isomer hypothesis.

As for the postulate that uranium daughter activity provides a source of polonium atoms, I agree that in special

cases polonium haloes can form in that manner: hundreds of such secondarily formed ^{210}Po haloes have been found² in a uranium-rich coalified wood from the Colorado Plateau. Earlier, when uranium-bearing solutions interacted with the uncoalified wood, numerous sites were formed containing a variety of elements. Uranium haloes subsequently developed around uranium-rich sites and ^{210}Po haloes around Pb–Se inclusions. The absence of ^{214}Po and ^{218}Po haloes means that even though the hydrothermal transport was high enough to permit the accumulation of ^{210}Po and ^{210}Pb to form ^{210}Po haloes, it was too low for many of the nuclides with shorter half-lives— ^{214}Pb , ^{214}Bi , ^{214}Po and ^{218}Po —to accumulate before decay. Thus, the formation of ^{214}Po and ^{218}Po haloes was inhibited.

Acceptable as that explanation is as far as ^{210}Po haloes in coalified wood are concerned, there are formidable obstacles in using it to account for the variety and distribution of ^{210}Po , ^{212}Po , ^{214}Po haloes in minerals. Except along conduits or cracks where solutions have passed, atomic migration in minerals proceeds by diffusion. Since the r.m.s. distance, x , that an atom of diffusion coefficient, D , will migrate in time, t , is $x = (2Dt)^{1/2}$ and the maximum value of D_{Pb} (at 20 °C) in 7 different minerals³ is $10^{-18} \text{ cm}^2 \text{ s}^{-1}$, a ^{210}Pb atom (with a half life of 22 yr) would migrate, on average, about 10^{-5} cm before decay. Even at 1,038 °C the D_{Pb} value ($10^{-11} \text{ cm}^2 \text{ s}^{-1}$) in microclineperthite³

yields negligible migration ($x = 0.11 \text{ cm}$) of a ^{210}Pb atom before decay. Further, this is a somewhat unrealistic case, for even though shortened fission tracks exist in some micas⁴, the great majority of minerals containing polonium haloes show no evidence of high temperature episodes. Concerning Po haloes in fluorite I can be very specific. The D_{Ba} ($\approx D_{\text{Pb}}$) value is less than $10^{-22} \text{ cm}^2 \text{ s}^{-1}$ at 300 °C (ref. 5) and it is certain that the fluorite has not exceeded that temperature during or since halo formation, because halo coloration disappears within minutes in this temperature range⁶. In that case a ^{210}Pb atom would migrate only about 10^{-7} cm before decay.

Thus, if Po or Pb radionuclides were migrating to Po halo radiocentres, most would have decayed in transit and produced a large excess of α -recoil tracks close to the radiocentres, which is contrary to observation⁷. An equally strong objection to the uranium-daughter hypothesis in uranium-poor (p.p.m. or less) minerals is that many Po haloes (such as the 'spectacle' halo) are located in the interior of large pegmatitic crystals as well as in small granitic mica flakes where they are often more than 10 cm and sometimes much less than 100 cm away from a significant uranium source. The fact that ^{214}Po and ^{218}Po haloes often occur profusely in some minerals (10^3 – 5×10^4 haloes cm^{-3}), whereas their formation was inhibited even in the uranium-rich (16% U) coalified wood where nuclide transport

rates were comparatively high, certainly suggests some other origin for polonium haloes in minerals.

But if isomers and uranium-daughter diffusion did not produce polonium haloes in rocks, we are left with the idea that polonium haloes originate with primordial Po atoms just as U and Th haloes originate with primordial ^{238}U and ^{232}Th atoms. Thus, a new type of Pb exists in polonium halo radiocentres, derived specifically from primordial polonium α -decay. Carried to its ultimate conclusion, this means that polonium haloes, of which there are estimated to be more than 10^{15} in the Earth's basement granitic rocks⁸, represent evidence of extinct natural radioactivity, and thus imply only a brief period between 'nucleosynthesis' and crystallisation of the host rocks.

If this conclusion is considered highly improbable, then another explanation for the origin of the polonium should exist.

Research supported by Union Carbide Corporation and ERDA, and by Columbia Union College with an NSF grant.

¹ Gentry, R. V., et al., *Nature*, **244**, 283 (1973).

² Gentry, R. V., et al., *Eos*, **56**, 473A (1975).

³ Keneshea, F. J., Jr., and Fredericks, W. J., *J. chem. Phys.*, **38**, 1951 (1963); Tilton, G. R., *J. geophys. Res.*, **65**, 2933 (1960); Nicolaysen, L. O., *Geochim. cosmochim. Acta*, **11**, 41 (1957); Sheshtakov, G. I., *Geochem. Int.*, **9**, 801 (1972); Rosenquist, I. T., *Acta chem. scand.*, **3**, 569 (1949).

⁴ Gentry, R. V., *Science*, **173**, 727 (1971).

⁵ Baker, M. E. thesis, Cornell Univ. (1968).

⁶ Schilling, A., *Neues Jb. Miner. Abh.*, **A53**, 241 (1926).

⁷ Gentry, R. V., *Science*, **160**, 1228 (1968).

⁸ Gentry, R. V., et al., *Nature*, **252**, 564 (1974).

Microorganisms on manganese nodules

We report here tubular forms found on the outer surface of a piece of coral coated with manganese from the eastern flank of the Mid-Atlantic Ridge (biotrawl at 747 m, V22-D6 Lamont–Doherty Geological Observatory). Scanning electron microscopy (SEM) of a dark zone of the coral revealed well preserved coiled tubular fragments which may be *Saccorhiza* averaging 2–3 μm in diameter (Fig. 1a–d). These tubular structures were not found on the inner surfaces. They are, however, ubiquitous throughout the dark ferromanganese zones on the outside of the manganese-coated coral. They seem merely to adhere or be cemented to zones rich in ferromanganese which show little distortion (Fig. 1b–c). We suggest that these organic structures are better preserved on fragments coated with ferromanganese than in the interior of compacted ferromanganese modules such as those studied by Greenslate¹.

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¹ Greenslate, J., *Nature*, **249**, 181–183 (1974).

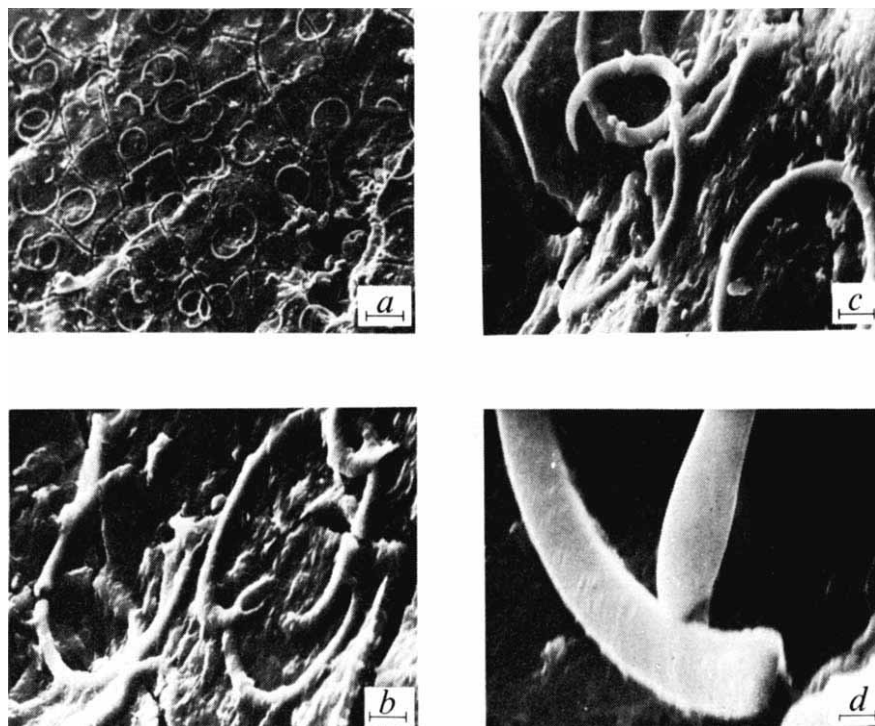


Fig. 1a, SEM of the outside region of manganese-coated coral (V22-D6), showing circular, tubular structures (scale bar, 80 μm); b and c, SEM of circular structures showing their adherence (b) and cementation (c) to the surface and their tubular nature (scale bar, 8 μm); d, SEM of tubular structures 2–3 μm in size (scale bar, 1.6 μm).

reviews

Outweighing bones of contention

By any standards, the life and career of Louis Leakey was extraordinary. Kenyan by birth, Kikuyu by adoption and anthropologist by education he was still more: contemporary historian, palaeontologist, graphologist, museum curator, Criminal Investigation Department investigator, zoologist. But in this age of specialisation he was no less remarkable for his diversification than for his accomplishments, as these two books amply demonstrate.

By *The Evidence*, the second instalment of Louis Leakey's memoirs, covers the years from 1932 to 1951 and follows the initial volume, *White African*, which dealt with his first thirty years. *By The Evidence* encompasses a period of remarkable and varied productivity but also of serious professional conflict and controversy. These are the years of the initial explorations at Olduvai Gorge, Rusinga Island and other sites, the first Pan-African Congress, the discovery of *Proconsul*; they are also the years of stinging criticism and debate over his claims for the fossil hominids from Kanam and Kanjera. This was also a period of active involvement with police investigations in Kenya and of the development and expansion of the Coryndon Museum in Nairobi into what eventually became the National Museum of Kenya. The book eloquently describes his concern for the vanishing cultures and languages of East Africa; it also documents his efforts and ultimate frustrations in preserving something of the past for the future. His three-volume, 700,000-word monograph on the Kikuyu is still unpublished, although funds from the Leakey Foundation may soon solve this problem.

By the Evidence: Memoirs, 1932-1951. By L. S. B. Leakey. Pp. 276+12 photographs. (Harcourt Brace Jovanovich: New York and London, January 1975.) £2.95.

Leakey's Luck: The Life of Louis Seymour Bazett Leakey, 1903-1972. By Sonia Cole. Pp. 448+50 photographs. (Collins: London, May 1975.) £5.50.

Louis with man-eating crocodile on Rusinga Island, Kenya



Sonia Cole's excellent book, *Leakey's Luck*, is a fine complement to the memoirs. Her close involvement with both the events and personalities she describes makes her book a particular delight. Moreover, her background in prehistory eminently qualifies her as both biographer and critic, and her book constitutes, in fact, a chronicle of studies of early man in East Africa. Although a small amount of fossil human material had been recovered there in the early years of this century, notably "Olduvai Man" by Hans Reck in 1913, it was Leakey's initial work in 1926 which marked the real beginnings of such studies in East Africa.

The controversy which followed so much of Leakey's life began with the report of the human material from Kanam and Kanjera, recovered during the third East African Expedition, in 1931. Leakey contended that the Kanjera skulls and the Kanam mandible represented evidence of an advanced type of human at a very early period in the Pleistocene. This controversy has yet to be finally resolved and his search for a morphologically more modern type of man in the early Pleistocene continued unabated throughout his life. It is ironic that whereas his own candidates for this group, which included *Homo habilis*, have not found wide acceptance in the field, his son Richard's discoveries at east Rudolf have been accepted, almost without equivocation, as very early members of the genus *Homo*.

But Louis Leakey's contributions to

the development of Palaeoanthropology as a discipline, far outweigh such "bones of contention", to use Mrs Cole's phrase. In 1947, he organised the First Pan-African Congress on Prehistory, which was held in Nairobi. This Congress represented an important turning point in the evolutionary study of man; not the least of its effects was to remove the focus of fossil man studies from Asia and to re-direct it to Africa where it has remained ever since. At the Congress in 1947 scientists from many different and yet inter-related disciplines met and discussed aspects of their own research involving human evolution in Africa. It was here that Le Gros Clark, in opposition to then widely held opinion, gave convincing arguments why the South African Australopithecines should be included within the human family. It was also at this time that the enormous potential and importance of the East African Miocene deposits became more widely appreciated. As a result of this Congress Leakey received a grant to continue his explorations in deposits of this age and in the following year Mary Leakey found the skull of *Proconsul* on Rusinga Island; to date, this remains the only Miocene hominoid skull known.

Both books are rewarding. *By The Evidence* is a unique and personal anecdotal remembrance, whereas *Leakey's Luck* provides a broad perspective on East African prehistoric studies.

G. E. Kennedy

Spectroscopy from end to end

Molecular Spectroscopy. By Ira N. Levine. Pp. x+491. (Wiley-Interscience: New York and London, June 1975.) £10.50.

Organic Spectroscopy. (A Macmillan Chemistry Text.) By William Kemp. Pp. xvi+248. (Macmillan: London and Basingstoke, June 1975.) £7.95 hard cover; £2.95 paper.

SPECTROSCOPY not only covers a wide frequency range but also has a wide range of interested practitioners from devoted theoreticians to organic chemists. These two books serve opposite ends of this range. Take the area of the ultra-violet spectroscopy of aromatic molecules. An organic chemist, hoping to identify and estimate a small amount of such material in solution before tea-time, would find *Organic Spectroscopy* a valuable reminder, or even introduction, for what is needed. But a PhD student with a research topic to identify the electronic structure and symmetry of an excited electronic state and determine quantitatively its origin, vibrational structure and molecular geometry could usefully study much of *Molecular Spectroscopy* before he starts and all before he faced his examiners.

Both are good books of their kind, although each carries a slight feeling of distilling the best parts of their predecessors rather than adding a new consistent approach. For instance Levine, who is admittedly re-writing parts of, and re-arranging, his own earlier book, uses something like a Dirac bra/ket nomenclature for matrix elements but avoids, except for a few equations in chapter 8, the simplicity its full use would bring. He also refers to SI but does not use these units and consequently uses the c.g.s. Gaussian equations rather than rationalised m kg s A versions and associated quantity calculus—as increasingly favoured in schools and essential for consistency with SI. The material seems scientifically sound and reliable and the coverage appropriate, including a new section on photoelectron spectroscopy, although optical rotation and circular dichroism are regrettably absent. I am continually embarrassed by my failure to answer my colleagues who ask “Can you give us a simple picture of what is happening to the molecule and its electrons as optical activity is observed?”

Kemp's book does include this topic briefly, as well as mass spectroscopy. The whole is written in simple language and seems to be appropriate for the bench chemists for whom it is intended, with the slightly more difficult parts identified for digestion at a second reading. The infrared section seems one of the best and I am rather less happy with the one on nuclear magnetic resonance, which

attempts to explain sophisticated ideas with a classical picture.

Both are well produced and value for money. **D. H. Whiffen**

Intimate knowledge of Wales

The Prehistoric, Roman and Early Mediaeval Field Monuments. By Christopher Houlder. Pp. 207+29 plates. (Faber and Faber: 1975.) £4.50.

FABER'S series of archaeological guides under the editorship of Glyn Daniel grows apace. To the British coverage, already including James Dyer's highly successful *Southern England*, the present volume makes a very welcome addition. It's a surprising fact that this is the first adequate guide to Welsh sites ever to be published.

The author divides the principality into eight regions, determined by the natural structure of the land, and assigns a chapter to each. Within the regional structure all the major sites the listed and briefly described. The text is liberally sprinkled with plans, illustrations of objects and a well chosen series of aerial photographs and includes good references and such tourist information as is necessary.

All that, and the accompanying short introductory sections containing information on museums, archaeological organisations, ordnance survey maps, suggested tourist centres and so on, make this an ideal handbook for visitors.

Where the book is a little thin (and this, one suspects, is no fault of the author) is in the actual body of archaeological background information given. Admittedly, there is a seven page outline of the archaeology of Wales which is a masterpiece of intelligent compression, and wherever possible ancillary information is slipped into the main text, but even so it will be difficult for the general user to relate the sites he visits to the development of human societies in the Welsh landscape. The general context would have benefited from a fuller treatment. For the more archaeologically inclined, the bibliography will be helpful and the simple cross referencing scheme will enable the reader quickly to find the principal published account of most sites.

Erratum. In the Book Reviews Supplement (*Nature*, 258, November 6, 1975), the reviewer of *The Seas* by Sir Maurice Yonge and Sir Frederick Russell (page 36) was in fact C. E. Lucas. We apologise for any inconvenience or embarrassment this error may have caused.

The author is to be congratulated in producing a succinct but invaluable guide to Welsh field archaeology—a distillation of his own intimate knowledge of the landscape and its early inhabitants. **Barry Cunliffe**

Nuclear magnetic resonance and biochemistry

Nuclear Magnetic Resonance in Biochemistry: Principles and Applications. By Thomas L. James. Pp. xii+413. (Academic: New York and London, June 1975.) \$26.50; £12.70.

It is now clear that nuclear magnetic resonance (NMR) studies can provide detailed structural, conformational, ionisation and kinetic information about large biological molecules, some of which is inaccessible using other techniques. Not surprisingly, many biochemists and biophysicists wish to learn more about the theory and application of the NMR technique. Dr Thomas' book is aimed directly at such readers who will undoubtedly find it useful reading. Starting from first principles, the text describes all the relevant techniques and develops the necessary NMR theory for tackling biological problems. Special emphasis is placed on the theory related to exchange and relaxation effects, which are the most important aspects for biological applications. The theoretical sections are clearly written and, for the most part, the reader should be able to see how the final parameters (such as exchange rates and correlation times) can be calculated from raw data (line widths and relaxation times). In a book of this type, however, it would probably have been useful to work through more examples from the raw data to the final results.

In the section on spin-spin splittings (pages 95–97), a confused definition of the signs of coupling constants is given and it is also incorrectly implied that 1,2-disubstituted ethanes give A_2B_2 -type spectra.

An extensive review is given of the applications of NMR to studies of polypeptides, proteins, protein-ligand interactions, membranes and water in biological systems. This section provides an interesting descriptive account of most of the applications to date. Although no attempt is made to evaluate critically the conclusions of the various papers, this part of the book (about 180 pages) constitutes a most useful reference source.

I can recommend the book to anyone interested in learning how to apply NMR to biological problems.

J. Feeney

Dictyostelium discoideum

Dictyostelium discoideum: a Developmental System. By William F. Loomis. Pp. x+214. (Academic: New York and London, May 1975.) \$19.50; £9.35.

Dictyostelium discoideum is undoubtedly one of the most intriguing and fascinating organisms that biologists and biochemists have sought to study. It can be cultured as separate amoebal cells showing no apparent cellular interaction and yet at will it can be induced to mass together and develop into a multicellular body that can move and be responsive to light and heat signals in its environment. It is also very good at taking punishment meted out by the curious investigator. It can be squashed, pricked, stained, drugged, decapitated or thoroughly dissociated to separate cells and still resurrect itself and proceed with its planned development. In this book Dr Loomis records and discusses the descriptive and analytical work of many such investigators.

In the early part of the book, the life cycle and various stages of the development are discussed, together with a review of some of the genetic experiments that have been carried out with the organism. After chapters detailing the analysis of cellular enzymes and macromolecular composition, the author then reviews the research concerned with the effects on development of metabolites and such agents as light, heat, cell density, mechanical barriers, dissociation and mutation. The book ends with a discussion of the ubiquitous biological problem of the control of tissue proportions and the various theories that might account for the surprising constancy of ratio of stalk cells to spores in *Dictyostelium*. Such a division into chapters concerned either with developmental stages or selected topics covering several stages must have caused problems for the author over the subdivision of information without causing undue repetition. Generally this difficulty has been skilfully overcome in this book and except for the topic of cell sorting out which is rather awkwardly divided between pages 69–71 and 169–170, the chapter arrangement is rational and helpful to the reader.

The book is written in a style that is lucidly descriptive and unambiguous. The writing lacks flamboyance or sparkle, but whether this is a point for praise or criticism depends on whether one wishes to be succinctly informed or entertained. The illustrations are plentiful and of good quality and include photomicrographs using Nomarski optics and scanning and transmission electron microscopy; they add a great deal to the interest of the book.

The account of the various experiments described in the text is, inevitably, some-

what subjective, although only occasionally is this tendency obtrusive. If any criticism is due, it is possibly to the occasional failure of the author to make it clear whether a particular statement is a considered review of the consensus of opinion or the author's own particular viewpoint.

The author notes in the preface that he has attempted to put into focus most of the major studies on *D. discoideum* and generally I feel that he has been successful. One area of research that might also have been included as a major study is that of the work on radiation effects by Dr R. A. Deering and his colleagues at Pennsylvania State University using normal and γ -ray-resistant strains of the organism. The bibliography which is fairly comprehensive is also lacking in many of the interesting papers from Dr Deering's laboratory.

In general this is an excellent book and I would recommend it to anyone wishing to acquire an overall impression of the past and current areas of research using *Dictyostelium*. Dr Loomis has carefully trodden the narrow path avoiding both superficiality and academic pendency, and has succeeded in producing a book that is both readable and thought provoking.

P. C. Newell

Astronomical applications of interferometer

The Intensity Interferometer: Its Application to Astronomy. By R. Hanbury Brown. Pp. xvi+184. (Taylor and Francis: London, November 1974.) £6.

THE original stellar intensity interferometer at Narrabri, New South Wales, finished its program in February 1972, and Professor Hanbury Brown has celebrated its achievements and looked forward to the future by writing this account of its construction and use. If only more research programs could be recorded in this way. Reading the book is to acquire twenty years of experience painlessly in a few hours. One can read how physicists proclaimed that the interferometer could not work; that the Australian customs officials were fought for a year and defeated; how the main steel tubes supporting the reflectors sagged; how the reflecting surface was accidentally stripped off; and how parrots pecked through the electric cables. Substitute appropriate personalities and pests and you have a story which could be adapted to describe the progress of many another astronomical project. In many ways the intensity interferometer was vulnerable and exposed to attacks of all sorts, even more so than most telescopes, because of the originality of the design and the rural surroundings. The lessons learned must be appropriate to other ventures.

This is not to say that one is reading a chapter of accidents; far from it, as the difficulties were overcome and most of the book contains a straightforward account of this successful work—for example, one has the full theory of the intensity interferometer and a choice of simpler explanations to suit one's taste. The technical description is given in considerable detail, although without an exact analysis of the system of lenses. The scientific results subsequently described show the power of the new technique. Although the primary program, to determine the angular diameters of stars and their emergent fluxes, was carried out, many other interesting possibilities have arisen from the studies of individual stars and multiple stars. If the reader hasn't already guessed, this leads to a proposal for a new instrument to extend and continue the programs. One is pleased to record that the second interferometer is going ahead and to wish its builders as much success and almost as much excitement.

R. G. Bingham

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Cytogenetics

Chromosomes and Cancer. Edited by James German. Pp. xxv+756. (Wiley: London and New York, November 1974.) £17.40.

It is well established that the great majority of cancer cells have grossly abnormal chromosome complements. Since malignancy is, in essence, a heritable loss of growth control, it is obviously tempting to relate its development to these visible derangements of the genetic material. Because some clearly malignant cells have apparently normal chromosomes, and because the chromosome abnormalities observed seem (with a few notable exceptions) to lack specificity, it has been argued that these microscopic rearrangements are merely a by-product or late result of the malignant process.

The book is the first in a series devoted to cytogenetics, although we are not told what other topics will be covered. It starts rather charmingly with a brief scientific autobiography by Curt Stern, followed by a review (by U. Wolf) of Theodor Boveri's 1914 publication *On the Problem of the Origin of Malignant Tumours* in which the questions of this field were first clearly formulated. The body of the book consists of what are essentially 22 review articles, grouped into four sections: disturbances of the genetic material; cancer as a clone; cytogenetics of certain specific cancers; and special approaches. The chapters stand pretty well independent of one another, with relatively little cross reference. This inevitably leads to some repetition—the classification of visible chromosome damage into gaps, breaks, and so on, is covered by three different authors. There is also some imbalance in the amount of space devoted to different topics—for example, Makino uses 31 pages (excluding references) to review in minute detail the admittedly fascinating but limited subject of canine venereal tumours, which are seemingly transmitted from animal to animal by whole-cell transfer, whereas H. J. Evans covers the entire field of radiation cytogenetics in only five pages more.

As with any multi-author book, styles vary widely. Personally I do not find this a disadvantage. The quality also varies quite considerably, from chapters which are rather dull catalogues of major publications to some really excellent resumés of complex subjects. I particularly enjoyed David Comings arguing for unisexuality—"What is a chromosome break?"—O. J. Miller's section on cell hybridisation, and S. M. Gartler's review of the use of biochemical markers in mosaic individuals (including females heterozygous

for X-linked markers) to study the clonal origin of tumours.

The recent and unexpected metamorphosis of the Philadelphia chromosome from a deletion into a translocation caught some authors with their hypotheses showing. In fact, the subject matter of the book by and large antedates the application of chromosome-banding techniques to tumour cytogenetics; and much new information is bound to emerge over the coming years. Dr German writes in his introduction that, for this very reason, it is "a highly suitable time to review previously accumulated knowledge". I thoroughly agree with him. Recent advances must be read in the current literature—a book like this justifies its cost by summarising, in digested and digestible form, a vast and complex literature which will generally be relevant for a good many years to come.

The general standard of contributions is high. I recommend this book to anyone interested in the subject of cancer, either for a quick overview of accumulated cytogenetic knowledge, or as a detailed reference source for those actively engaged in the field.

Martin Bobrow

Defects in solids

Theory of Defects in Solids: Electronic structure of defects in insulators and semiconductors. (Monographs on the Physics and Chemistry of Materials.) By A. M. Stoneham. Pp. xix+955. (Clarendon: Oxford; Oxford: London, May 1975.) £29.25.

THIS is a particularly worthy book, one which has long been needed by theoretician and experimentalist alike. Approximately the first half of the book leads one through a very detailed discussion of all aspects of the necessary theory, stressing the role of the perfect lattice, the electronic structure of the defect, and lattice dynamics. All the major theoretical models are discussed and their shortcomings made clear, in some cases brutally so. I think that the solid-state theorist will enjoy this part of the monograph particularly, although for the beginner it will be a daunting task. Many theorists will also find the second half of the book invaluable as the comparison of experiment and theory is especially well presented. Not only will it be informative, but it may imbue the reader with the motivation, so evident in all Stoneham's work, to attempt calculations of direct applicability to experiments already done and still to be done. This last part of the book will be much used by the experimentalist, not simply "to know what, if anything,

they should believe of the present theories" but also because it will give them a clearer vision of the interaction between theory and experiment in solid-state physics.

There are various areas of defect physics which are not treated as they do not fall within the remit of the author to concentrate the electronic properties of defects in insulators and semiconductors. Thus, there is no attempt to treat line and planar defects, nor mechanical properties and defect production mechanisms. If this restricts the readership then the price will do so even more. Such possible restriction is a pity as this is an important book of considerable scholarship.

B. Henderson

Treatise on Solid State Chemistry. Volume 2: Defects in Solids. Edited by N. Bruce Hannay. Pp. xiii+527. (Plenum: New York and London, 1975.) \$42.00.

THIS volume is the second in a series of works which aim to present the properties of solids from a chemical viewpoint. Studies of defects, imperfections or impurity ions may be described equally well from a basis of chemistry or physics and this volume has a definite chemical bias but is still suitable for physicists or materials scientists. As the existing literature on defects is predominantly oriented towards physicists it is refreshing to read a book with this change of emphasis. Within the volume seven aspects of defect properties have been selected and each is presented by a different author. Chapter titles range over the following topics: electronic structure and spectra of impurities in the more ionic crystals; colour centres in ionic crystals; dielectric properties; transport properties; semiconductors; magnetic properties; mechanical properties. In a single volume such a range of separate contributions has led to some differences in the level of presentation but in general the book is suitable for first year postgraduates or people who are unfamiliar with some aspect of solid state chemistry. It is not easy to generalise on the work of seven authors but I felt that the book was clear and informative in those areas with which I am least familiar. In more familiar areas, however, it was apparent that much of the book must have been written several years ago. For an introductory text this is a minor fault but one consequence is that the references tend to be rather old and in an active field such as solid state chemistry this is a pity. Similarly in a few cases defect structures are discussed in terms of outdated models. In spite of these limitations this volume on defects in solids should prove a useful text.

P. D. Townsend

Principles of Microbe and Cell Cultivation. By S. John Pirt. Pp. x+274. (Blackwell Scientific: Oxford and London; Halstead: New York, 1975.) £10.50.

THIS book has been written for students of microbiology and as a reference book for research workers involved in the problem of microbial cell growth. The treatment of the subject is a clear simple mathematical one which should enable the reader readily to understand the dynamics of the different techniques of cell cultivation.

The book is divided into 25 chapters with an appendix of the symbols and abbreviations used. The initial chapters are devoted to an historical outline and to a consideration of the general parameters of cell growth. Batch and continuous culture methods of cell growth are then discussed. The next four chapters deal with the supply of energy and oxygen to growing cells. The other factors affecting growth, general nutrition, temperature, pH and water activity are dealt with in separate chapters. There are a number of chapters of interest to microbiologists involved in the production of materials by microbial processes, for example, product formation, growth lag, and batch cultures with substrate feeds.

The subjects of interest to microbial ecologists are dealt with in chapters on slow growth rates, mixed cultures, and growth on surfaces as microbial films. Finally, growth of colonies on solid media, and mathematical models of biomass autotrophy are dealt with.

Microbial growth is usually treated as a biochemical phenomenon and many excellent textbooks are available which use this approach. The dynamics of cell growth, however, are usually given only superficial treatment; thus, Professor Pirt's book fills a much needed gap in the microbial literature. But both approaches have their merits and both types of book are needed to obtain a balanced viewpoint.

It would have been useful to widen the discussion a little more to show how the ideas outlined could be applied to specific problems. On several occasions the primary references to the subject are not given.

Overall Professor Pirt's book is a very useful addition to the microbial literature and should be read by all those interested in the dynamics of microbial and cell cultivation.

D. C. Ellwood

The New Mars: Discoveries of Mariner 9. By William K. Hartmann and Odell Raper. Pp. 179. (NASA Office of Space and Science: Washington, DC, 1974.) \$8.75.

THIS is in many ways the definitive book to emerge from the Mariner 9 adventure, produced with the cooperation of the Mariner 9 Science Exploration Team. As such it provides a well-written insight into one of the most successful scientific endeavours undertaken by man, and should be of interest to many people outside the circle of specialist astronomers and space scientists. For the price, it is worth having just to look at the lavish illustrations; serious planetary astronomers will not gain much from the text that has not already been published, but they may find the general overview of Mars useful, together with the bibliography provided in an appendix.

John Gribbin

Books brief

Interfacial Electrochemistry: An Experimental Approach. By E. Gileadi, E. Kirowa-Eisner and J. Penciner. Pp. xviii+525. (Addison-Wesley: Reading, Massachusetts and London, June 1975.) Cloth \$19.50; paper \$13.50.

THIS book is divided into two sections, theoretical and practical. The theoretical section (150 pages) is a brief account of basic interfacial electrochemistry with three chapters covering corrosion, energy conversion and electroplating. The theoretical chapters succeed in the authors' aim of providing the fundamentals of electrode kinetics and double-layer theory; the other three chapters, however, are inadequate and could well have been omitted.

The rest of the book is an excellent advanced practical text. Fourteen chapters describe current techniques of electrochemistry. The arrangement of the subject matter is good. Starting with the basic electronics (emphasis on op-amps) and reference electrodes, the reader is led through the majority of the important techniques of electrode kinetics and interphase studies applied to well defined systems. There are a few criticisms,

ring-disc electrodes are omitted and impedance methods are not given sufficient space; electrometallurgy and electrocrystallisation are not dealt with. But these are of small importance, the main references are there at the end of each chapter and the reader is guided by the Authors to the major works of electrochemistry.

The book is a valuable addition to the literature of electrochemistry, and the price is reasonable by contemporary values.

N. A. Hampson

Normal Values in Clinical Chemistry: a Guide to Statistical Analysis of Laboratory Data. (Clinical and Biochemical Analysis: A Series of Monographs and Textbooks, Vol. 2.) By Horace F. Martin, Benjamin J. Gudzinowicz and Herbert Fanger. Pp. viii+504. (Marcel Dekker: New York, May 1975.) \$37.50.

MORE and more pathology laboratory results, especially those of clinical chemistry, are numerical in form. Clinicians are accustomed to interpreting such results with the aid of the normal range, a range of values intended to include those to be expected from a large proportion (conventionally 95%) of healthy individuals. This is clearly a statistical concept, and the statistical problems of estimating the limits of the normal range have received a good deal of attention. In fact these problems are quite straightforward; the real difficulties are logical ones, notably in deciding on what is meant by the "normal" population, and practical ones in obtaining the necessary data.

This book attempts to provide the clinical chemist with the statistical techniques needed to assess normal limits, but the attempt is far from successful. A very large literature, not all of it relevant, is extensively surveyed, but the uncritical approach and lack of logical organisation makes it almost impossible to follow the authors' drift. Explanations of the basic statistical principles vary from obscure to downright misleading. Even the practical problems are quite inadequately handled; it is never quite decided whether or not normal limits can be derived from data relating to hospital patients, and the large effects of the menopause on female biochemistry are not mentioned. Clinical chemists should wait for a better book on the subject, which should be considerably shorter and less expensive.

M. J. R. Healy

announcements

Awards

The Royal Society has announced the following awards: The **Copley Medal** to **F. H. C. Crick** for his elucidation of the structure and function of DNA and his continuing important contributions to molecular biology; The **Davy Medal** to **T. M. Sugden** for his contributions to physical chemistry including particularly the analysis of the reactions occurring in flames; The **Hughes Medal** to **R. H. Dalitz** for his contributions to the theory of the basic particles of matter; The **Leverhulme Medal** to **F. L. Rose** for his contributions to the application of chemical science to industry.

International meetings

January 5-6, **Aspects of water use**, Loughborough (John Pickford, University of Technology, Loughborough, Leicestershire, UK).

January 5-7, **Solid State Physics Conference**, Manchester (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK).

January 5-9, **Chemistry and biology of peptides**, California (Gordon Research Conferences, Pastore Chemical Laboratory, University of Rhode Island, Kingston, Rhode Island 02881).

January 6, **Photorespiration**, London (Dr B. Halliwell, Biochemistry Department, King's College, The Strand, London WC2, UK).

January 6-9, **Research and medical practice**, London (The Ciba Foundation, 41 Portland Place, London W1N 4BN, UK).

January 12-16, **Minicomputers/microprocessors and their applications to real world problems**, Zurich (Industrial Liaison Office, Room 39-615, MIT, Cambridge, Massachusetts 02139, USA).

January 12-16, **Fermentation technology**, Zurich (Industrial Liaison Office, Room 36-615, MIT, Cambridge, Massachusetts 02139, USA).

January 12-16, **Proteolysis and physiological regulation, and cancer enzymology**, Miami (Miami Winter Symposia, PO Box 520906, Florida 33152, USA).

January 21-22, **Energy-brake or break**, London (Institute of Fuel, 18 Devonshire Street, Portland Place, London W1N 2AU, UK).

January 22, **Volcanic processes in ore genesis**, London (Dr I. L. Gibson, Volcanic Studies Group, Bedford College, London NW1, UK).

January 26-30, **Biology of aging**, California (Gordon Research Conferences, Pastore Chemical Laboratory, University of Rhode Island, Kingston, Rhode Island 02881).

Miscellaneous

Data on Cytogenetic Registers. The International Advisory Committee on Cytogenetic Registers to the Standing Committee on Standardisation in Human Cytogenetics is compiling, updating and publishing a list of cytogenetic registers, together with summaries of aims, objectives and key elements. The Committee has developed a form for the collection of such data and invites individuals who operate Cytogenetic Registers (either in isolation or as part of a larger register system) to write to Dr. James R. Miller, Department of Medical Genetics, The University of British Columbia, Vancouver, B.C., V6T 1W5, Canada.

Reports and publications

Great Britain

Department of the Environment, Scottish Development Department, Welsh Office. 111th Annual Report on Alkali, &c. Works 1974. Pp. vii + 134. (London: HMSO, 1975.) £2.25 net. [2010]

Ministry of Agriculture, Fisheries and Food. Report of the Director of Marine Fisheries Research, April 1973/March 1974. Pp. 63. (Lowestoft, Suffolk: Fisheries Laboratory, 1975.) [2010]

Patent Cooperation Treaty—Minimum Documentation. By Brenda M. Rimmer. (A guide to the Patent Documents and Journals Currently Proposed for Inclusion). Pp. 24. (London: The British Library, 1975.) £1. [2110]

British Museum (Natural History). Guide for Young Visitors. Pp. 30. (Publication 773.) Bulletin of the British Museum (Natural History). Zoology. Vol. 28, No. 6: The Hydroid Species of *Obelia* (Coelenterata, Hydoroza: Campanulariidae), with Notes on the Medusa Stage. By P. F. S. Cornelius. Pp. 249-293. £2.50. Vol. 28, No. 7: Some New and Rare Species of Calanoid Copepods from the Northeastern Atlantic. By H. S. J. Roc. Pp. 295-372. £4.75. (London: British Museum (Natural History), 1975.) [2110]

British Nutrition Foundation. Annual Report, 1974/1975. Pp. 28. (London: British Nutrition Foundation 93 Albert Embankment, SE1, 1975.) [2210]

Memoirs of the Royal Astronomical Society. Vol. 80, Part 1: Accurate Positions and Identifications for a Complete Sample of 341 Radio Sources from the Parkes + 4° Survey. By N. J. McEwan, I. W. A. Browne and J. H. Crowther. Pp. 1-59. Vol. 79, Part 2: Stromgren Four Colour Observations of Northern Hemisphere Binary Systems. By R. W. Hilditch, and Graham Hill. MK Classifications of Some Northern Hemisphere Binary Systems. By Graham Hill, R. W. Hilditch, Frank Younger and W. A. Fisher. Pp. 101-129. (Oxford and London: Blackwell Scientific Publications, 1975. Published for the Royal Astronomical Society.) [2210]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 280, No. 1293: A Discussion on the Early Days of Ionospheric Research and the Theory of Electric and Magnetic Waves in the Ionosphere and Magnetosphere. Organised by W. J. G. Beynon, FRS and J. A. Ratcliffe, FRS. Pp. 1-224 + plates 1-11. (London: The Royal Society, 1975.) UK £10.50; Overseas £11. [2310]

Introducing MRPA—Descriptive Brochure. Pp. 32. (Brickendonbury, Hertford: The Malaysian Rubber Producers' Research Association, 1975.) gratis. [2310]

Ministry of Agriculture, Fisheries and Food. Fishery Investigations. Series II, Vol. 27, No. 8: The Yorkshire Fishery for Edible Crabs (*Cancer pagurus*)—Assessment of the Effects of Changes in the Minimum Legal Size. By D. A. Hancock. Pp. iv + 11. 65p. Series II, Vol. 27, No. 9: Marine Fish Cultivation—Pioneering Studies on the Culture of the Larvae of the Plaice (*Pleuronectes platessa* L.) and the Sole (*Solea solea* L.). By J. E. Shelbourne. Pp. iv + 29. £1.80. (London: HMSO, 1975.) [2310]

ICI Information Handbook 1975-6. Pp. 48. (London: Imperial Chemical Industries, Ltd., 1975.) [2310]

The British Library—Science Reference Library. Recent Books on Polymer Science and Technology. Pp. 16. (London: The British Library, Store Street, WC1, 1975.) [2310]

Annual Report of the Scottish Marine Biological Association for the year ending 31st March, 1975. Pp. 63. (Oban, Argyll: Scottish Marine Biological Association, 1975.) [2410]

Ministry of Agriculture, Fisheries and Food. Agricultural Development and Advisory Service. Pest Infestation Control Laboratory, Technical Bulletin 31: Insect Travellers. Vol. 1: Coleoptera. By Audrey D. Aitken. Pp. xvi + 191 + 12 plates. (London: HMSO, 1975.) £4.24 net. [2410]

You and Your Back. By Dr. David Delvin. Pp. 34. (Teddington, Middx.: Back Pain Association, Somerset Road, 1975.) 25p. [2710]

Meteorological Office. Annual Report 1974. Pp. xiv + 129. (London: HMSO, 1975.) £1.80 net. [2710]

Ministry of Overseas Development. Annual Report of the Directorate of Overseas Surveys for the year ended 31st March 1974. Pp. 62 + 5 maps. (London: HMSO, 1975.) £2.40 net. [2710]

University of Oxford, Oration by the Vice-Chancellor and Annual Report, 1974/1975. (Suppl to *Oxford University Gazette*). Pp. 59-78. (Oxford: The University, 1975.) 20p. [2710]

International Planned Parenthood Federation. Annual Report for 1974. Pp. 68. (London: International Planned Parenthood Federation, 18/20 Lower Regent Street, SW1, 1975.) [2810]

The Institution of Gas Engineers. Discussions of the 112th Annual General Meeting, London, 1975. Pp. 136. (London: The Institution of Gas Engineers, 1975.) £1.50. [28910]

Thames Water Annual Report and Accounts for the year ended 31st March, 1975. Pp. 82. (London: Thames Water Authority, New River Head, Roseberry Avenue, EC1, 1975.) £1. [2910]

Agricultural Development and Advisory Service. Key for the Field Identification of Apterous and Alate Cereal Aphids with Photographic Illustrations. (Pinner, Middx.: Ministry of Agriculture, Fisheries and Food (Publications), Tolcarne, Drive 1975.) £2.10. [3010]

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 271, No. 914: The Jumping Mechanism of *Xenopsylla cheopis*. By Miriam Rothschild, J. Schleim, M. J. Cullen, K. Parker, C. Neville and S. Sternberg. Pp. 457-520 + plates 25-43. (London: The Royal Society, 1975.) UK £6.10; Overseas £6.30. [3010]

Other Countries

Office de la Recherche Scientifique, et Technique Outre-Mer, Paris, Systématique et Phylogénèse des *Spardiidae* du Genre *Diplodus* RAF. (Pisces, Teleostei). Par Reynaldo de la Paz. (Travaux et Documents de l'ORSTOM, No. 45.) Pp. 96. (Paris et Bondy: ORSTOM 1975.) 30 francs. [210]

International Development Research Centre, Ottawa. Projects 1975. Edited by Claire Veinotte. (IDRC-047e). Pp. 56. (Ottawa: International Development Research Centre. PO Box 8500, 1975.) [210]

United States Department of the Interior: Geological Survey. Water-Supply Paper 2029-E: An Appraisal of Potential Water Salvage in the Lake McMillan Delta Area, Eddy County, New Mexico. By E. R. Cox and J. S. Havens. Pp. ix + 26. \$1.50. Professional Paper 437-G: Land Subsidence Due to Ground-Water Withdrawal in the Los Banos-Kettleman City Area, California, Part 3. Interrelations of Water-Level Change, Change in Aulifer-System Thickness and Subsidence. By William B. Bull, and Joseph F. Poland. Pp. ix + 62. Professional Paper 818-C: The Effect of Sedimentation and Diagenesis on Trace Element Composition of Water-Laid Tuff in the Keg Mountain Area, Utah. By David A. Lindsey. Pp. iv + 35. Professional Paper 847: Quaternary Stratigraphy and Extent of Glaciation in the Mount Rainier Region, Washington. By Dwight R. Crandell and Robert D. Miller. Pp. vi + 59 + 2 plates. \$1.60. Professional Paper 892: Physical Results of Research Drilling in Thermal Areas of Yellowstone National Park, Wyoming. By D. E. White, R. O. Fournier, L. J. P. Muffer, and A. H. Truesdell. Pp. iv + 70. Professional Paper 940: Mineral Resource Perspectives 1975. Pp. v + 24. (Washington: DC: Government Printing Office, 1974 and 1975.) [210]